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The Effects of Homogenized Cream and Commercial Buttermilk Powder on Low-Fat Cheddar Cheese.

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THE EFFECTS OF HOMOGENIZED CREAM AND COMMERCIAL BUTTERMILK POWDER ON LOWFAT CHEDDAR CHEESE

A Dissertation

Submitted to the Graduate Faculty of the
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Agricultural and Mechanical College
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requirements for the degree of
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in

The Department of Dairy Science

by
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ABSTRACT

Seventy percent reduced fat Cheddar cheese was manufactured with homogenized cream and sweet cream buttermilk powder in an effort to improve flavor and texture characteristics. Manufacturing procedures, as well as the chemical, physical and sensory attributes. Pasteurized cream was homogenized (15.8 MPa) with and without the addition of buttermilk powder. The cream was added to pasteurized skim milk to produce two vats of cheese, one with and one without buttermilk powder. After milling, each vat was split to produce a washed curd and normal curd cheese. The four cheeses were sampled at week one, and months one, two and four. Both buttermilk cheeses had a stronger cheddar flavor, described as sulfide, by the fourth month. There was no significant difference in the percent of citric acid pH 4.6 soluble nitrogen between control and treatment cheeses. The percent of soluble nitrogen increased over time for all groups. Gel electrophoresis failed to identify fat globule membrane proteins from the buttermilk powder in the 4 month old cheeses. Membrane proteins were found in the wheys of both treatment and control cheeses. Analysis of the free fatty acids was conducted on the extracts of the cheese by solid phase extraction and gas chromatography. No significant differences were noted. Reversed phase HPLC of the citric acid and pH 4.6 soluble peptide fractions identified one peak as having a consistently greater area in the buttermilk cheese. Sensory analysis was conducted on lowfat cheese made with homogenized cream and lowfat cheese made with homogenized cream and buttermilk powder. An experienced panel of 8 conducted attribute analysis. Control cheeses were firmer, more crumbly and more curdy than the buttermilk cheeses. Buttermilk cheeses were more bitter, acid, sulfide, unclean and had a stronger flavor than the control. Consumer evaluations were conducted at 2, 3 and 4 months of aging.

Buttermilk cheese had a significantly softer texture in the first panel at two months of aging. The control cheese was preferred for flavor and overall liking after three and four months of aging.

CHAPTER 1: INTRODUCTION

Cheddar cheese is the natural cheese produced in the greatest quantity in the United States. Almost 2.5 million pounds are produced each year (National Cheese Institute, 1994). The second most abundant varieties are those used in pizza making, like mozzarella, of which about 2 million pounds are produced. The legal definition of Cheddar cheese is that it has a fat content of 50% by dry weight and a maximum moisture of 39% (Food and Drug Administration, 1995). For a 28g (1 oz) serving, this amounts to 110 calories, 73% (9 g) of which are from the fat. The relatively high fat content of cheese, in general, is therefore one important reason why reduced calorie dairy products are second only to diet beverages in American consumer reduced calorie popularity (Barr, 1990). The large amount of fat contained in Cheddar cheese is the result of the traditional process used to produce the cheese by the controlled removal of water from milk. The milk is usually standardized to a fat content of approximately 3.2%. A typical cheese yield of 10% is expected and since most of the fat is contained in the cheese, the final product has at least 32% fat on a wet basis.

Attempting to produce Cheddar cheese from milk with a lower fat content with the same methods as the full fat product results in a cheese that is too firm and rubbery and lacks typical Cheddar flavor. During cheese production, the proteins form a network which entraps milkfat either physically or chemically (Olson and Johnson, 1990). The surface of the milkfat globule is an especially important factor in this interaction (van Vliet and Dentener-Kikkert, 1982). The final cheese consists of "islands of fat" trapped in the protein matrix (Olson and Johnson, 1990). Fat contributes to flavor by its ability to act as a solvent for flavor compounds produced by the hydrolysis of fat, protein and other compounds (Ardö, 1997; Olson and Johnson,

1990). Fat is also thought to mask bitter flavors (Ardö, 1997). The exact mechanism by which fat contributes to texture is unclear. The amounts of water in the non-fat substance and the protein per unit volume have the greatest effect on cheese firmness, elasticity, and adhesiveness (Olson, 1984; Olson and Johnson, 1990).

Simple changes in manufacturing can produce acceptable cheeses with a one-third fat reduction. Greater reductions, however, result in an inferior product. Consequently, most of the nationally marketed reduced fat natural Cheddar cheeses have only a one-third reduction in fat. Non-fat cheeses are available but these are process cheeses consisting of melted curd, added emulsifiers, and other ingredients. These products bear little resemblance to the natural cheese product. To produce an acceptable cheese with a greater than one-third fat reduction, it is necessary to combine changes in the traditional process with ingredient additions. Three main strategies for improving lowfat cheese have been pursued. These strategies include modifications of the cheesemaking procedure, the use of different strains of traditional starter organisms and adjunct cultures, and the use of fat replacers.

Cheesemaking procedures are the most simple and inexpensive steps to modify. These modifications usually focus on the problems of too much acid development and the need of increased moisture content to improve the texture. For unknown reasons, the pH at the time of draining the whey and salting should be higher than normal to produce a better lowfat cheese (Olson and Johnson, 1990). The higher pH will result in less chymosin being retained in the cheese which might help to reduce chymosin produced bitterness (Olson and Johnson, 1990). Increased pH should also result in greater incorporation of plasmin, a natural milk protease important in flavor intensity (Olson and Johnson, 1990). Shortening the time the lactic culture ripens, the duration of cheddaring, and selecting a slow growing lactic culture strain, can lessen acid development. The major culture suppliers have developed slow growing, mesophilic

Lactococcus lactis spp. *cremoris* strains specifically for low fat Cheddar cheese to help address these concerns.

Higher moisture contents are thought to help reduce the effect of greater amount of protein per unit area in the lowfat cheese which results in firmness (Anderson *et al.*, 1993). Normally fat globules are physically entrapped in the protein matrix and provide lubrication and break-up of the network. The moisture in non-fat substance (MNFS), which describes the ratio of water to protein, is a good predictor of cheese firmness in cheeses with less than a one-third fat reduction (Emmons *et al.*, 1980). Reduced fat cheeses can be manufactured with similar texture to full-fat cheeses if the MNFS is similar in both cheeses. This does not hold true, however, for greater than one-third fat reductions (Olson, 1984). The cooking stage is a very important step in determining the final moisture content of the cheese. Reducing the final cooking temperature from the typical 39°C to 37.8°C, and reducing the time held at this temperature from 30 min to zero results in curds that will retain more moisture. Reduced cooking temperature will, however, lower the plasmin activity since the precursor to plasmin is activated by high temperatures (Ardö, 1997). Another factor in the process important for moisture retention is the cheddaring step. Increasing the size of the blocks at cheddaring and reducing the time of cheddaring will retain more moisture in the blocks (Drake and Swanson, 1995).

Homogenization of milk used in cheesemaking lowers fat losses in the whey, reduces oiling-off and increases fat hydrolysis (Johnson, 1988). The homogenization process disrupts the fat globule membrane and breaks the globules up into smaller globules. Their re-coalescence is prevented by caseins adsorbing to the surface. Fat hydrolysis is good for blue cheese manufacture and the physical effects of homogenization are beneficial for spreadable cheeses like cream cheese and Neufchâtel. However, homogenizing the milk for Cheddar cheesemaking has adverse effects. It can

seriously effect the formation of cheese curd. Homogenization causes caseins to adsorb to the surface of the fat globule because there is not enough fat globule membrane to cover the greatly increased surface area of the smaller globules (Johnson, 1988). The casein associated with the fat surface is not able to associate with other caseins to form a gel as quickly or as strongly. As a result, the setting time is longer, and the gel is weak and retains more moisture (Johnson, 1988; Kosikowski, 1990). Homogenizing only the cream portion of the cheesemilk and adding this back to the cheesemilk has been shown to improve lowfat cheese texture (Mayes *et al.*, 1994; Metzger and Mistry, 1994). This process increased the moisture content in these cheeses but did not adversely affect coagulation. Increasing the surface area of the fat is important since the fat interface is thought to be important for flavor development and texture (Olson and Johnson, 1990).

Washing the curd of the cheese after milling is a modification that can adjust the moisture content and can decrease acidity. Washing the curd decreases acidity both by removing lactic acid and some lactose, which would be available for starter cultures to metabolize (Olson and Johnson, 1990). When curds are washed with water at a temperature less than the cooking temperature, they will absorb moisture and approximately 2 % more moisture can be incorporated (Kosikowski, 1977). When curds are washed with water at a temperature higher than cooking temperature, they will dehydrate and lose moisture.

The lactic starter cultures desirable for lowfat cheeses have less proteolytic activity than those used for full-fat cheese and slower acid development as mentioned previously (Ardö, 1997). These modifications can result in a cheese that lacks flavor. One possible approach to address this problem is to add enzymes with specific aminopeptidase activity to the system. This can be accomplished by the use of adjunct cultures. *Lactobacillus* and *Micrococcus* species, both important non-starter

microorganisms in Cheddar cheese have been investigated for their effects on lowfat cheese (Drake and Swanson, 1995; Lee *et al.*, 1992). Another option is to add cell-free extracts, or heat or cold shocked cells or mutants of these organisms to improve the flavor without increasing the acidity (Lee *et al.*, 1992).

Fat replacers have been used in cheese to simulate the properties of fat. These replacers fall into two categories, those that possess the same functional properties of fats and oils and those that bind water in the product. The replacers with the same functional properties as fat include those based on sucrose fatty acid polyesters, like Olean (Proctor and Gamble, Cincinnati, OH), and structured lipids, like Caprenin (Proctor and Gamble, Cincinnati, OH) and Salatrim (Nabisco Foods, East Hanover, NJ). Drake *et al.* (1994) synthesized sucrose polyesters from milkfat and incorporated these into reduced and lowfat Cheddar cheese. These researchers noted an improvement in texture, but flavor problems were attributed to the fat substitute. Fat mimetics, or those replacers that primarily bind moisture in the product, are mainly protein or carbohydrate derivatives. Dairy protein mimetics include Dairy Lo (Pfizer, Inc., Groton, CT), and Simplesse (Nutrasweet Co., Deerfield, IL). Novagel (FMC Corp., Philadelphia, PA) is a carbohydrate based fat mimetic. These products are polar and help bind moisture in the product. They can not, however, act in the same capacity as fats non-polar functions such as in flavor carrying and in their chemical role (Drake and Swanson, 1995).

An ingredient that has been studied for a long time for its inclusion in reduced fat cheeses is buttermilk. Buttermilk is the waste product of buttermaking. When cream is churned, the fat globule membrane is sheared off and the contents of the globule, mainly triacylglycerols, start to coalesce. The fat granules increase in size until a phase inversion is reached and the cream, which was an oil in water emulsion, becomes butter granules and buttermilk. The buttermilk is drained, the granules are rinsed, and

churning continues until the homogeneous water in oil emulsion, or butter, is achieved. The buttermilk then can be condensed and dried. Buttermilk powder has a composition very similar to nonfat dry milk powder except that it has as a higher fat content and more fat globule membrane material. About 50% of the membrane is released into the buttermilk during churning (Swaisgood, 1985). It is the milk fat globule membrane (MFGM) that has sparked the attention of various researchers to include buttermilk in reduced fat dairy products. It is generally accepted for the MFGM material to have originated from both the endoplasmic reticulum and the apical membrane of the mammary secretory cell (Keenan *et al.*, 1988). As such, it contains enzymes, proteins and phospholipids that might contribute to the improvement of lowfat cheese. Enzymes could generate flavor volatiles, proteins could generate novel peptide flavors, and phospholipids could emulsify the fat globules as well as provide a source of flavor from their unsaturated fatty acids. The exact composition of the MFGM is difficult to determine because of the various ways of purifying the membrane. The membrane must be washed to remove adsorbed contents of the globules, but not washed too much so as to remove loosely bound, yet real membrane components (Keenan *et al.*, 1988). Enzymes associated with the membrane include xanthine oxidase, alkaline phosphatase, sulfhydryl oxidase, phosphodiesterase, and plasmin (Swaisgood, 1985). Xanthine oxidase is the most abundant enzyme of the membrane. It is thought to interact with lacto-peroxidase in milk to produce oxidizing agents that oxidize lipids and destroy microorganisms (Richardson and Hyslop, 1985). Sulfhydryl oxidase catalyzes the oxidation of thiols to produce disulfides and hydrogen peroxide (Richardson and Hyslop, 1985). This enzyme could be an important addition in Cheddar cheese where sulfur flavors are important in the aged product. About 40% of the total phospholipids in milk are located in the MFGM. Phospholipids are natural emulsifiers that promote oil in water emulsions (Nawar, 1985). The most abundant phospholipids in milk are

phosphatidylcholine (lecithin), phosphatidylethanolamine (cephalin), sphingomyelin, phosphatidylinositol, phosphatidylserine, and lysophosphatidylcholine. These phospholipids compose relatively 36, 27, 22, 11, 4 and 2% of the total phospholipids, respectively. Phosphatidylcholine and phosphatidylethanolamine contain 40 - 60% unsaturated fatty acids, one third of which are polyunsaturated (Deeth, 1997). This high degree of unsaturation makes these phospholipids very susceptible to oxidation. Fat globules tend to associate to lessen their surface free energy (Swaisgood, 1985). Homogenizing the cream to be used in the milk with added phospholipids could improve the fat dispersion in the cheese. Drake and Swanson(1995) reported adding lecithin to reduced fat Cheddar cheese. Texture was improved but the flavor was foreign and undesirable. The MFGM may effect how the fat globules interact with the protein matrix in cheese and effect elasticity. Van Vliet and Detener-Kikkert (1982) washed fat globules to remove the membrane material. These globules did not interact with the protein matrix in acid milk gels formed and did not contribute to elasticity. In contrast, globules coated with casein micelles by homogenization did contribute greatly to elasticity. El Soda (1997) reported a study that indicated more than 85% of the starter cells are located at the peripheral region of the fat globule when viewed by electron microscopy. They appeared to interact with the MFGM and the membranes appearance altered during ripening. This could be a reason for the noting by researchers of the importance of the fat/water interface for flavor in cheese (Foda *et al.*, 1974). The MFGM accounts for 2 to 3% of the weight of the milk fat globule yet it contains 10% of the cholesterol of the globule (Kosikowski, 1990). This could be a detriment to its use in a lowfat product since often people consume reduced fat animal products to consume less cholesterol. Cholesterol is reduced during the manufacture and aging of cheese, however (Kosikowski, 1990). It would be important to monitor this when using MFGM material in reduced fat products.

Buttermilk addition to cheese was first reported by Marinskiy in 1940. He reported the addition of buttermilk to skim milk to produce a lowfat, aged cheese (Marinskiy, 1940). In 1966 Madsen *et al.* compared the effects of adding fresh buttermilk, reconstituted buttermilk, and reconstituted skim milk in a lowfat brick-type cheese . These researchers found increases in firmness and flavor defects with all of the treatments when compared to the control. They concluded these ingredients were not useful for adding to lowfat cheese at 20 - 30 % additions. Law *et al.* (1973) investigated the addition of fat globule membrane material to Cheddar cheese. These researchers manufactured their own buttermilk from buttermaking and freeze-dried it. Its inclusion to cheesemilk was compared with control milk, and milk to which butteroil was added to simulate conditions with no membrane material. Lipolysis was reported in the cheeses made with butteroil. The butteroil cheesemilks were homogenized, whereas the buttermilk ones were not, and the homogenization process itself could have increased the lipolysis of the fat. Law and his co-workers (1973) concluded this but also attributed it to the lack of MFGM, even though the homogenization of the cheesemilk with MFGM was never conducted. The researchers concluded that MFGM material was not important for flavor, but that a lack of MFGM resulted in lipolysis. El-Sadek *et al.* (1969) manufactured full-fat baby -Edam with 20, 40 and 60% buttermilk additions. The cheeses with buttermilk added had significantly higher moisture and protein on a dry matter basis but had significantly less fat. These researchers also stated that the cheeses with 20 and 40% buttermilk additions were superior in taste, flavor, aroma, and texture by organoleptic evaluations. Foda *et al.* (1974) investigated the role of fat in the flavor of Cheddar cheese by using various fat sources and milkfat with and without MFGM. These researchers homogenized the fats into the cheesemilks. The fat and water interface was concluded to be important for flavor in this experiment because cheeses having either MFGM or gum acacia (an emulsifier) emulsified into them were

able to develop Cheddar flavor whereas those without them did not. Mayes *et al.* (1994) added buttermilk manufactured from butter churned in a batch churn to lowfat Cheddar cheese. These researchers removed the buttermilk portion from the churn. Buttermilk was also obtained by melting the manufactured butter and separating the remaining aqueous phase from the butter by gravity. This was added back to the bulk of the buttermilk. The addition of buttermilk to lowfat Cheddar cheese, with and without homogenizing it into the cream portion of the cheesemilk, was then investigated in this study. It was concluded that homogenization on its own made more of a difference on flavor and texture than buttermilk addition alone. The researchers did report the highest preference scores for cheeses both having buttermilk powder and homogenized cream. Mistry *et al.* (1996) investigated the use of ultrafiltered sweet buttermilk on the production of reduced fat Cheddar cheese. Improvements in the texture with the addition of the buttermilk were reported. The free oil expressed from cheeses with buttermilk added was less than that of the control. These researchers attributed this to the emulsification properties of the MFGM.

Drake and Swanson(1995) suggested that with fat reductions greater than 50%, modified methods should be combined. A combination of process changes, adjunct cultures and fat replacers is needed to develop a lowfat Cheddar cheese with desirable flavor and texture properties. In light of the contradictory reports on the use of buttermilk in cheesemaking, and in light of properties of buttermilk that would appear to improve attributes in lowfat cheeses, the research for this dissertation was undertaken. Approximately 14.5 million kg (32 million pounds) of buttermilk powder are utilized yearly by the dairy industry (Milk Industry Foundation, 1994). The bulk of this is used as a source of solids in the ice cream industry. None of the studies conducted on the use of buttermilk in cheese have utilized a commercial source of buttermilk powder. It would seem prudent to utilize this commercial source of high quality powder in the

production of a product destined to be produced by factory methods. This dissertation is, therefore, an attempt to modify the cheesemaking process with the addition of commercial buttermilk powder to improve the flavor and texture of lowfat Cheddar cheese.

CHAPTER 2: PRODUCTION OF LOWFAT CHEDDAR CHEESE

Introduction

Lowfat Cheddar cheese has recently become the focus of intense research with the goal to provide a healthier alternative for consumers interested in lowering their fat consumption. Removing the fat from traditionally manufactured Cheddar cheese has a detrimental effect on both flavor and texture. Fat content effects the mouthfeel, texture and flavor of cheese. Reductions in fat greater than 30% of the full fat counterpart, generally result in cheeses that are too firm and rubbery and either lack flavor or have an abnormal flavor.

Improvements can be made through culture selection, modifications to the traditional make procedure and reductions in the cooking time and temperature. This retards whey expulsion of the curds and increases the moisture content of the cheese (Anderson *et al.*, 1993). Washing the cheese curd improves the texture by removing lactose, hence reducing the developed acidity in the cheese (Anderson *et al.*, 1993). Homogenization of milk fat used to standardize the milk for cheesemaking has been noted to improve texture of reduced fat Cheddar cheese (Mayes *et al.*, 1994). This may be due to the increased moisture content in the cheese or the better dispersion of fat and fat globule membrane materials. Buttermilk powder contains increased levels of fat globule membrane materials but is otherwise very similar to skim milk powder. The unsaturated fatty acids of phospholipids are prone to oxidation and may enhance the lipid flavors in lowfat cheese (Law *et al.*, 1973). One study involving buttermilk addition to lowfat cheese used condensed, fluid buttermilk (Mistry *et al.*, 1995). An improvement in cheese body but a decline in flavor acceptability with aging was noted. Law *et al.* (1973) and Mayes *et al.* (1994) incorporated buttermilk powder, which they

had manufactured, into Cheddar cheese. Mayes *et al.* stated that the improvement they found in texture was mainly due to homogenization of the cream and any improvement in flavor from added buttermilk powder would not justify its cost. Neither study used a commercial source of buttermilk powder. In commercial buttermilk powder production, buttermilk is often held in bulk tanks until enough accumulates for the making of powder. It can be re-pasteurized and pre-condensed during this time before it is spray dried. This greatly affects the properties of the proteins in the powder. In this study, the addition of a commercial source of sweet cream buttermilk powder, homogenized into the cream portion of cheesemilk, was investigated as a way of incorporating membrane material and oxidizable lipids.

Materials and Methods

Preparation of Cheese Milk

High temperature short time pasteurized skim milk was purchased from a local dairy plant in 5 gallon bags and weighed into two vats in the amount necessary to standardize the cheesemilk to 0.9 % fat after homogenized cream addition. The amount of pasteurized cream required to standardize the skim milk in each vat, plus skim milk for diluting the cream for homogenization was weighed into two cans. The amount of skim milk added for diluting was two times the weight of the cream. For the buttermilk cheese, the buttermilk powder contained 4 % fat and this was computed into the standardization equation. Extra grade spray process dry sweet cream buttermilk powder (California Milk Producers, Artesia, CA) was added (1% on a weight basis) to the proper amount of diluted cream and mixed well. Both the cream containing buttermilk powder and the control were homogenized at 15.8 MPa on a two-stage homogenizer and added to the vats (12.4 MPa on the first stage, 3.4 MPa on the second).

Production of Cheese

The cheesemilk in the vats was brought to 32.3°C and 1% Redi-set RFC 300 culture specifically for reduced fat Cheddar cheese was added (Chr. Hansens's Laboratory, Inc., Milwaukee, WI). The vats were ripened for 45 min. Calcium chloride was added at a rate of 0.02 % (by weight). The vats were set with Chy-Max II fermentation produced chymosin (Pfizer, Inc., Milwaukee, WI) for 30 min. Curd was cut with 3/8 in knives, allowed to rest for 5 min and then brought to 37.8°C over 30 min with gentle stirring. After draining, trenching and knitting, curd was cheddared for one h and then milled at a titratable acidity of approximately 0.2%. Next, the curd from each vat was divided into two equal portions. Half was washed by soaking in 21.1°C water for 15 min before being drained and salted. This is considered the "Washed Curd" treatment in this study. The other half was salted normally. This is considered the "Normal Curd" treatment in this study. The rate of salting was 1.27 kg salt per 453.6 kg cheesemilk. The curds were hooped in 20 lb Wilson hoops and pressed overnight at 275.8 kPa. Blocks of cheese were removed from hoops and cut into 20 blocks. The blocks were vacuum sealed in moisture impermeable packaging and aged at 6°C. Cheesemaking was replicated 3 times.

Compositional Analysis

Total protein in the cheese was determined utilizing the Kjeltec-Kjeldahl method (Tecator, Hoeganas, Sweden). Moisture was determined by an atmospheric drying oven method (Richardson, 1985)). The amount of salt in the cheese was determined by a chloride ion selective electrode method for cheese (Orion Research, 1995) . This method was run on an Orion EA 940 ionanalyzer with a 94-17 chloride electrode and a 90-02 double junction reference electrode (Orion Research, Inc., Cambridge, MA). Ash was determined using a muffle furnace and the AOAC (1984) method for cheese 16.267. Fat in dry matter (FDM) was calculated as (% fat / (100 - % moisture)) x 100.

Moisture in nonfat solids (MNFS) was calculated as (% moisture / (100 - % fat) x 100. The percent of salt in moisture was calculated as (% salt / % moisture) x 100.

Electrophoresis

The presence of fat globule membrane proteins was investigated using SDS-PAGE on 15 cm vertical 1.5 mm thick gels employing 12 % and 15 % acrylamide running gels with 4% acrylamide stacking gels. Pre-weighed acrylamide crosslinked 2.6 % with bisacrylamide was used (Biorad, Richmond, CA). Extracts were prepared by mixing weighed protein or peptide samples with 1 ml sample buffer, vortexing and then heating at 95°C for 5 min. The amounts ranged from 0.02 - 0.2 g, depending on whether the sample was a freeze dried standard, cheese or whey. Ten ml of sample buffer consisted of 5 ml water, 1.25 ml 0.5M Tris at pH 6.8 [77-86-1](Biorad, Richmond, CA), 1 ml glycerol [56-81-5](EM, Gibbstown, NJ), 2 ml 10 % sodium dodecyl sulfate (w/v)[151-21-3](Biorad, Richmond, CA), 0.5 ml ß-mercaptoethanol [60-24-2](Biorad, Richmond, CA) and 0.25 ml 0.05 % bromophenol blue (Mallinckrodt, Paris, KY). Fifteen µl of sample were applied to each lane and run for approximately 4 h. When two gels were run, the power supply was set at 20 mA while samples were in the stacking gel and then was increased to 40 mA when they reached the running gel. Gels were stained with Coomassie Brilliant Blue R250 (Amresco, Solon, OH) and de-stained in methanol:acetic acid:water (5:1:4).

Peptide Analysis

Extracts were prepared by homogenizing 10 g of cheese with 40 ml 0.5 M trisodium citrate [6132-04-3](Sigma Chemical Co., St. Louis, MO) and 70 ml 40-50°C distilled water in an industrial blender for 8 min at the highest speed. Samples were cooled to 20°C, brought to 150 ml with distilled water, and filtered with Whatman #4 filters (Whatman, Fairfield, NJ) in a Büchner funnel with a water aspirator. Forty ml of filtrate were adjusted to pH 4.6 with 6M HCL. Samples were centrifuged for 30 min in

a Sorvall centrifuge at 7,710 G (Sorvall, Norwalk, CT). Supernatant was removed by decanting and was frozen until analysis. Prior to analysis, the thawed extract was filtered through a 0.2 μ membrane filter. Extracts were analyzed for percent nitrogen by using the Kjeltec-Kjeldahl method. The extracts were also analyzed by high performance liquid chromatography (HPLC) using a 250 x 4.6 mm Hypersil ODS 5μm reversed phase column (Sigma Chemical Co., St. Louis, MO). This was based on the method described by Belitz and Kaiser (Belitz and Kaiser, 1993). One-hundred fifty μl of sample was injected with a Wisp autosampler (Waters, Milford, MA). The column flow rate was 2 ml/min. A gradient elution was run using two Waters 501 pumps controlled by a Waters System Interface Module and gradient program from Waters Baseline 810 software (Waters, Milford, MA). The initial solvent was prepared by adding 5.025 ml of 1.0 M triethyl ammonium formate [585-29-5](Fluka Chemie Ag, Buchs, Switzerland) and approximately 800 ml HPLC grade water acidified to pH 4.6 with 0.1 M formic acid [64-18-6](Sigma Chemical Co., St. Louis, MO). Fifty ml acetonitrile [75-05-08](BandJ Brand, Muskegon, MI) was added and then the solution was brought to 1 L with HPLC grade water. The second solvent was prepared by adding 5.025 ml triethyl ammonium formate to approximately 150 ml HPLC grade water. The gradient went from 100 % initial solvent to 80 % second solvent over 60 min using a linear gradient. The solvent was then brought to 100 % second solvent over 5 min with a convex gradient and held at 100% second solvent for five min to clean the column. Next, the solvent was brought back to 100 % initial solvent in a linear gradient over 5 min. The column was held at 100 % initial solvent for 10 min before the next injection to allow the column to re-equilibrate. The column temperature was 60°C and the peptides were detected at 220 nm on a Waters 486 tunable absorbance detector.

Fatty Acid Analysis

One g of cheese was ground with 3 g anhydrous sodium sulfate with a mortar and pestle to help remove moisture. Samples were transferred to a test tube and 0.3 ml 2.5 M sulfuric acid and 1 ml internal standard solution were added. Internal standard solution consisted of 500 mg/L C_5, C_7, C_{13}, C_{17} (n-valeric acid [109-52-4], oenanthic acid[111-14-8], tridecanoic acid [638-53-97], and heptadecanoic acid [506-12-7] all from Fluka (Fluka Chemie Ag, Buchs, Switzerland). Three ml ethyl ether/heptane (1:1 v/v)[60-29-7][142-82-5](Mallinckrodt, Paris, KY) were added to the tube and vortexed. The tubes were centrifuged to clarify the solution. The solvent layer was removed and the extraction was repeated two more times. The extracts were stored at -40°C until they were extracted by solid phase extraction (SPE). Supelclean LC-NH_2 3 ml solid phase extraction tubes (Supelco, Bellefonte, PA) were conditioned twice with 5 ml heptane on a SPE vacuum manifold. Sample extract was applied to the column and filtered through dropwise under very low vacuum. Neutral lipids were eluted with 4 ml chloroform/2-propanol (2:1 v/v)[67-66-3](Mallinckrodt, Paris, KY). Fatty acids were collected by applying 2 ml ethyl ether with 2% formic acid twice to the tubes. Fatty acids were then analyzed by gas chromatography on a Hewlett Packard 5890 gas chromatograph with a flame ionization detector. One µl was injected by a Hewlett Packard 7673 GC/SFC injector. The sample was injected in split mode at 200°C. The carrier gas was helium at a flow rate of 12 ml/minute and a head pressure of 80 kPa. The column used was a Supelco Nukol 0.53 mm ID GC column (Supelco, Bellefonte, PA). The chromatography program started with the column at 125°C and ramping at 9°C/minute to 210°C. The temperature was held at 210°C for 10 min before ramping back to the initial temperature. Fatty acids were quantified by running standard solutions containing internal standards and analyzing with Waters Maxima 820 software, version 3.0

(Waters, Milford, MA). Fatty acids quantified were C_2, $C_{:4}$, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, and $C_{18:1}$.

Microbiological Analysis

Cheese was analyzed for total lactic acid bacteria by plating on Lactobacilli MRS agar (Difco, Detroit, MI). Cheeses were sampled at 0, 1, 2, and 4 months. Samples were mixed by a Stomacher in 0.1% peptone water and serial dilutions made in peptone water. They were then plated on MRS agar and were incubated anaerobically in a BBL Gas Pak culture system (Becton Dickinson Microbiology Systems, Inc., Cockeysville, MD) for 48 h at 35°C.

Texture Analysis

The texture of samples was analyzed on an Instron Universal Testing Machine 4200 series and Instron Series IX Automated Materials Testing System software version 1.08 (Instron Corporation, Canton, MA). Samples were equilibrated for 12 h in the environmentally controlled testing room at 21.1°C before testing. Samples were cut into 1 cm cubes with a thin wire cheese slicer immediately before testing. Samples were compressed with a 25 mm compression anvil obtained from Instron. No effort was made to prevent movement or friction. It was determined initially that this load cell could not receive a force sufficient to compress the samples to a break point. Samples were therefore compressed on a 10 N load cell at a load rate of 2 mm/min until the maximum load of 1 kg was reached or until the samples were compressed to 60 % of their original height.

Statistical Analysis

Statistical analysis was conducted using SAS for Windows ver. 6.11 (SAS, Cary, NC). The design was a split plot with blocking on the cheesemaking replicate. The vat of cheese with or without buttermilk was considered the whole plot and the washed curd or normal curd treatment was the subplot. The whole plot by replicate

interaction was used as the error term for testing the whole plot treatment effect. For analyzing proteolysis, moisture, free fatty acids, texture by Instron, and lactic acid bacteria, samples were taken one week, one month, two months and four months after cheesemaking. This time factor was treated as a repeated measure. Differences were considered significant when $\alpha < 0.05$.

Results and Discussion

Compositional Analysis

Adding buttermilk powder and homogenizing it with the cream caused the resulting cheese to have a significantly higher moisture content and a higher percent moisture in nonfat solids (Table 1). The washed curd and normal curd differed in moisture content with the normal curd cheeses having a significantly higher moisture than the washed curd treatments. Kosikowski (1977) stated that when the temperature of curd washing was less than the cooking temperature, the curd should retain approximately 1 to 2% more moisture than unwashed curd. In the current experiment, the temperature of washing was less than the cooking temperature yet the washed curd cheeses had less moisture than the normal curd cheeses. The reason for this is not known. Possibly this reported effect on moisture does not hold true in a reduced fat product, or in a cheese with homogenized cream. The washed curd treatments had blander flavors, particularly less acid flavor, than their normal curd counterparts. Mistry *et al.* (1996) and Mayes *et al.* (1994) both found increased levels of moisture in cheeses with added buttermilk powder. In the study by Mayes *et al.* (1994), buttermilk powder was also homogenized into the cream and resulted in a greater percent moisture than control homogenized cream cheese. This result was confirmed in the present study. The pressure of homogenization in the Mayes *et al.* study (1994) was much less than the pressure used in the present study. This could account for the higher total moisture and moisture in nonfat solids values which were observed in the current study, when

Table 1. Average percent composition of lowfat Cheddar cheeses.

Treatment[2]	Fat[1] (%) n=24	Moisture (%) n=96	Protein (%) n=24	Ash (%) n=24	Salt (%) n=24	Fat in Dry Matter (%)	Moisture in Nonfat Solids (%)	Salt in Moisture (%)	pH at 2 Months Aging
Control Normal Curd	9.8	49.9a[3]	33.7	4.5	1.8	19.6	55.3a	3.6	5.3
Control Washed Curd	10.3	49.3b	33.9	4.6	1.9	20.2	54.9a	3.9	5.3
Buttermilk Normal Curd	9.7	51.0c	32.0	4.4	1.9	19.7	56.5b	3.6	5.1
Buttermilk Washed Curd	10.3	50.5d	32.4	4.4	2.2	20.7	56.2b	4.4	5.2

1. Percentages are on a wet basis unless otherwise stated.
2. Treatment = Control is homogenized cream lowfat Cheddar. Buttermilk is homogenized cream lowfat Cheddar with 1% buttermilk powder added. Normal curd is the normal milling to salting procedure. Washed curd is the washing of the milled curds prior to salting.
3. Means with different letters indicate a significant difference ($p < 0.05$).

compared to Mayes *et al.* (1994). Lawrence *et al.* (1973) stated that a good quality full fat Cheddar cheese should have MNFS of 52 - 56 percent. The control cheeses fell into this range while the buttermilk cheeses were slightly higher. There was no significant difference in the percent protein between treatments when it was converted to a dry basis to account for the moisture differences. No significant differences were observed between the cheese treatment means for ash, salt, fat, salt in moisture, fat in dry matter, or pH.

It is notable that when grated cheese samples were dried for the moisture tests, the buttermilk treatments were light brown whereas the control treatments were light cream to yellow. This difference was observed throughout the aging period. Browning in cheese is usually attributed to an interaction between lactose and caseins. Thomas (1969) stated that the use of milk powder in process cheese is a factor attributed to browning in that product. Buttermilk powder is very similar to milk powder and could, therefore, have affected the cheese in a similar manner. The amount of lactose was not measured but can be assumed to decrease greatly throughout aging while it is used as a food source by the starter cultures. The mechanisms of this browning reaction, and it's persistence over time, would be interesting to study further.

Electrophoresis

The 15 % acrylamide gel of cheese aged for four months is shown in Figure 1 while the 12% acrylamide gel of day old cheese and whey is shown in Figure 2 . The 15 % gel was run initially to compare casein fragments more easily in the aged cheeses. Having a higher acrylamide percentage allows the smaller molecular weight fragments to be resolved better because the effective pore size is decreased and larger proteins will not be able to run on the gel (Hames, 1990). The 12 % gel allowed better viewing of the membrane proteins because they consisted mainly of high molecular weight fragments. The buttermilk powder differed from skim milk powder in having a high molecular

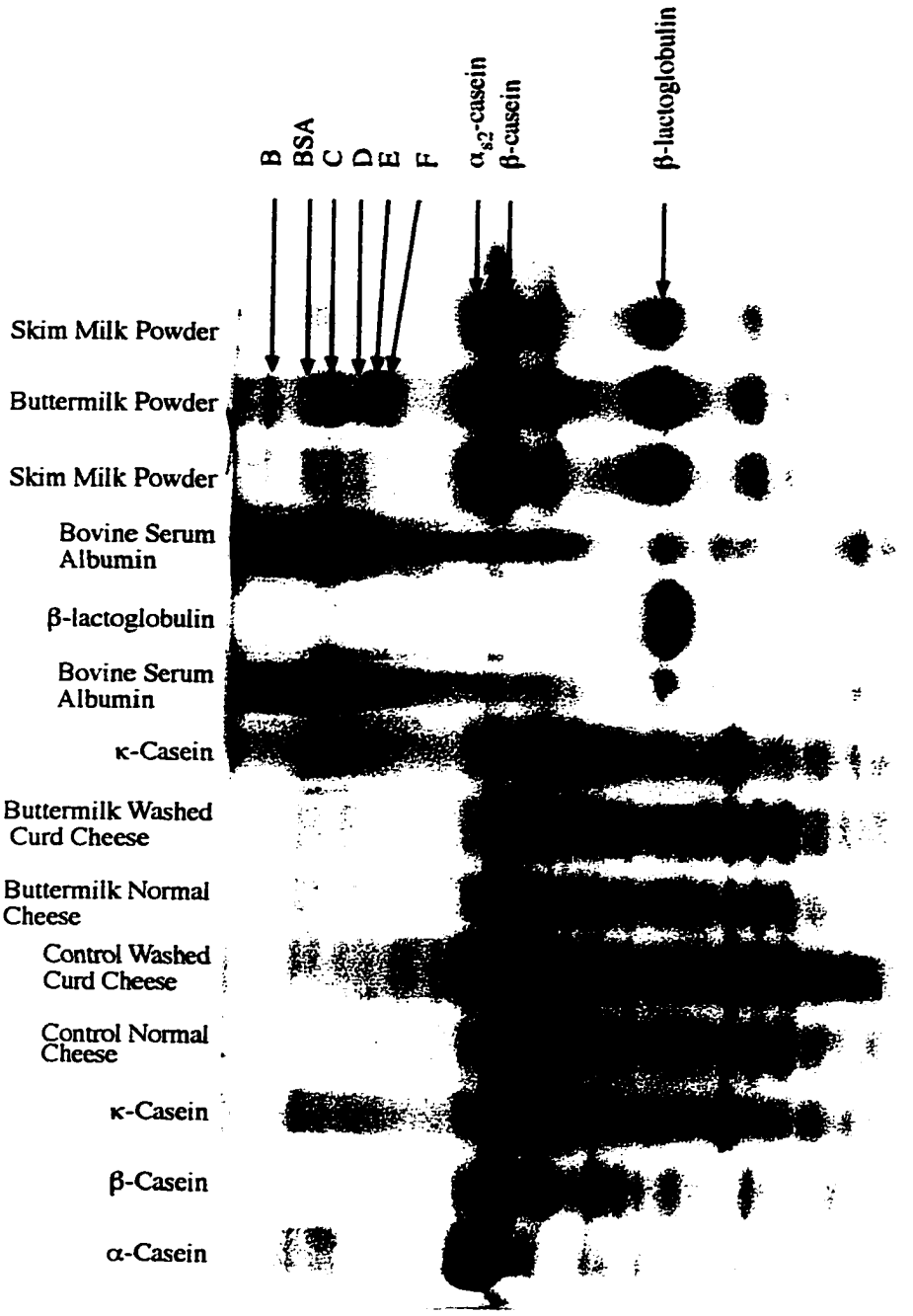

Figure 1. 15% SDS-PAGE of four month old control and buttermilk homogenized cream lowfat Cheddar cheese.

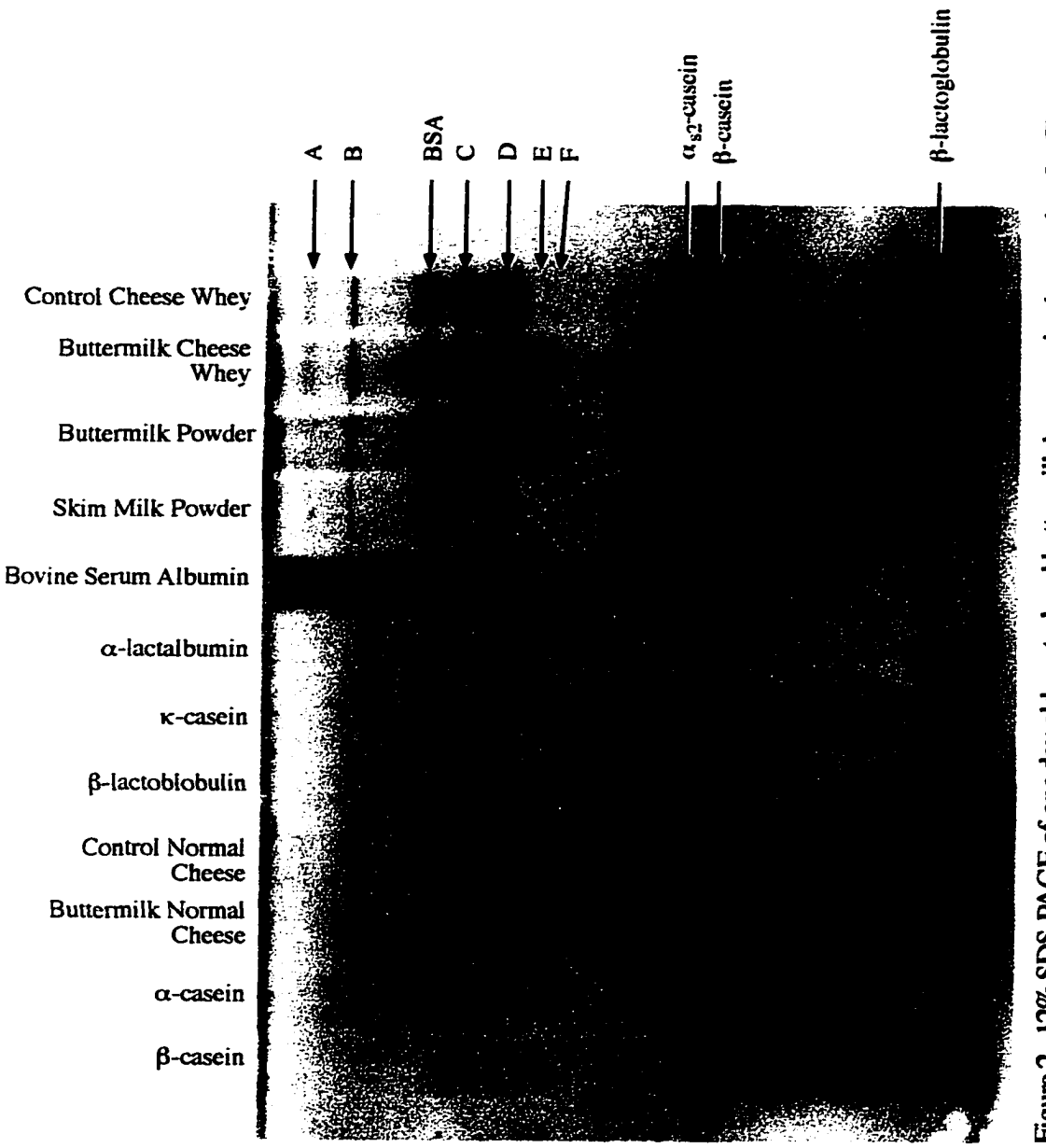

Figure 2. 12% SDS-PAGE of one day old control and buttermilk homogenized cream lowfat Cheddar cheese and whey.

weight protein (B) that also appeared in the bovine serum albumin (BSA) standard in a small amount. This protein is estimated to have a molecular weight of approximately 70,000 kD based on a standard curve of the log molecular weight versus Rf of standards at two gel concentrations. Bovine serum albumin itself is also present in slightly greater levels in the buttermilk powder than in the skim milk powder. Four more proteins C, D, E and F are found to run between BSA and the caseins in buttermilk powder. These proteins are estimated at molecular weights of 54,000, 48,500, 45,000 and 43,000 kD, respectively. Immunoglobulins, like IgG, are glycoproteins consisting of two heavy protein chains ranging in molecular weight from 50,000 - 70,000 kD and two light chains approximately 20,000 kD (Whitney, 1988). Two iron binding glycoproteins in milk, lactoferrin and transferrin have both been reported to be in the molecular weight range of 75,000 - 77,000 (Whitney, 1988). It is possible that the unknown proteins could be heavy immunoglobulin protein chains or iron binding proteins. Keenan *et al.* (1988) reported in a review that most studies of fat globule membrane proteins noted bands at molecular weights estimated at 48,000 and 44,000 kD. Bands D and E could be the same as noted in those other studies. Results of the one day old buttermilk cheese show membrane protein B whereas neither the one day old control nor the four month old cheeses contained it. Membrane proteins were observed to be mainly present in the cheese wheys. Both the control and buttermilk cheese wheys contained the membrane proteins B, C, and D. The control whey does not appear to contain proteins E and F whereas the buttermilk whey does. It appears that more of the caseins were lost in the buttermilk cheese whey than in the control cheese whey. This is most likely due to the increased solids in the cheesemilk by the addition of the buttermilk powder. One band of protein barely visualized in the 12 % gel is a high molecular weight protein that was only found in the cheese wheys and has an estimated molecular weight of 81,000 kD.

Peptide Analysis

The results of Kjeldahl analysis of citric acid pH 4.6 soluble extracts indicated no significant differences between treatments. There was a significant linear increase in the percent nitrogen of the extracts throughout the aging period (Figure 3). Chromatograms by HPLC of the afore-mentioned extracts were similar throughout aging. In both the control and buttermilk treatment cheeses, two large peaks were visible in the day old cheese extracts at 56.0 and 56.8 min, but were almost completely absent by month one (Figure 4, peaks "a" and "b")(Table 2). One peak in the buttermilk chromatograms was consistently larger, although not significantly, in the buttermilk cheeses than the control cheeses in all reps and regardless of curd treatment at four months (Figure 5, peak "a") (Table 3). This peak may be due to increased enzyme activity on a specific protein. A plasmin-like protease has been associated with the fat globule membrane and could have been added to the buttermilk cheese, although no differences were noted between the percent nitrogen of extracts from the treatments.

Free Fatty Acids

The fatty acid results are summarized in Table 4. A typical chromatogram of free fatty acids from cheeses aged 4 mo is shown in Figure 6. There were no significant differences between the treatments for any of the fatty acids. There was a significant difference over time for C_2, C_4, C_6, C_{10}, C_{14}, C_{16}, C_{18}, and $C_{18:1}$. This change with time was not linear, however. The initial concentration of the fatty acids was low and generally increased over the first two months of aging. By four months, the levels had decreased again, presumably due to being converted to other flavor compounds (Adda *et al.*, 1982; Olson and Johnson, 1997). Law *et al.* (1973) reported that fat globule membrane material was not involved in flavor development in Cheddar cheese. These investigators found no differences in the rancidity of cheeses with added freeze dried buttermilk by monitoring butyric acid. These findings are confirmed in this study.

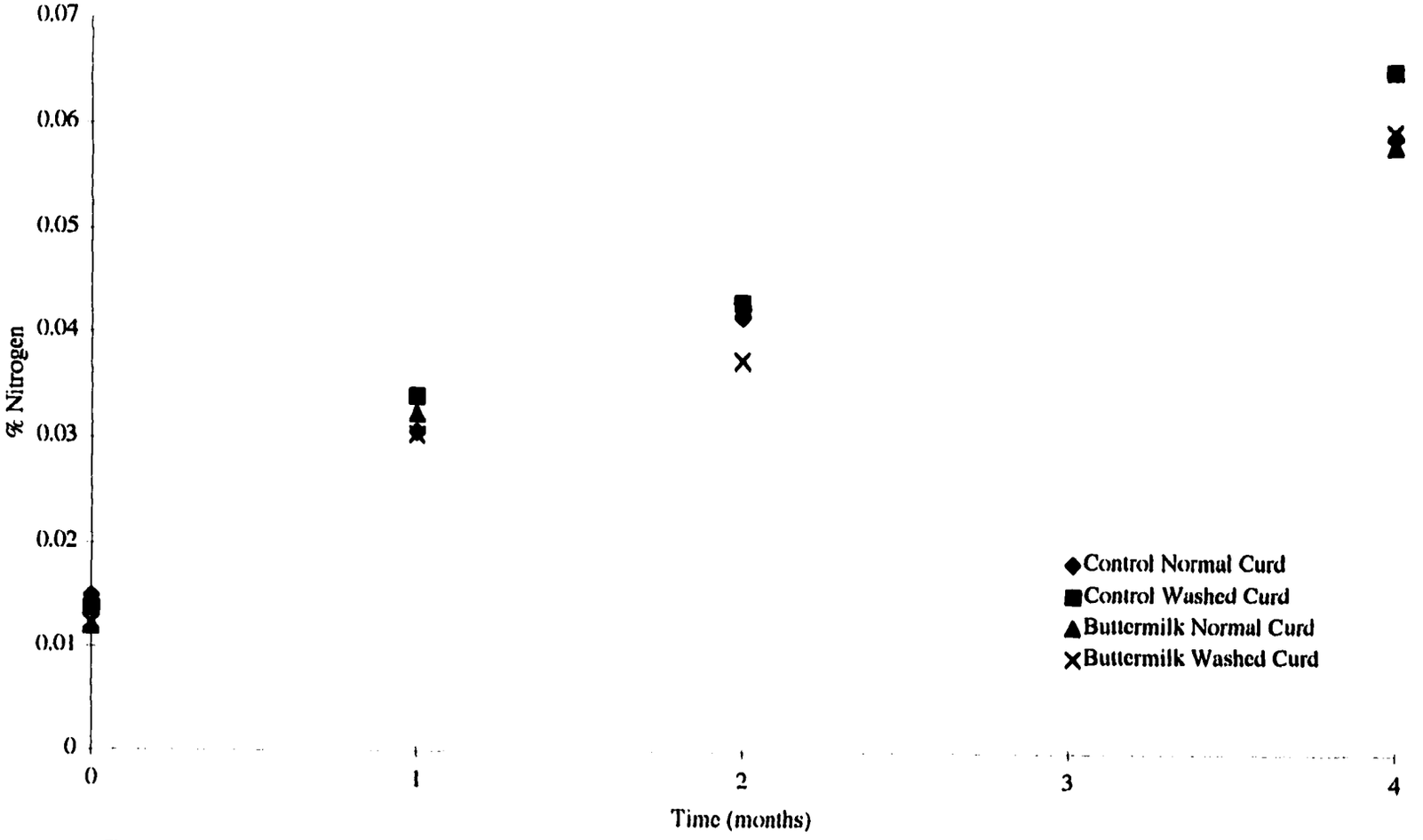

Figure 3. Mean percent Kjeldahl nitrogen in citric acid - pH 4.6 soluble extracts of lowfat Cheddar cheese aged at 6°C.

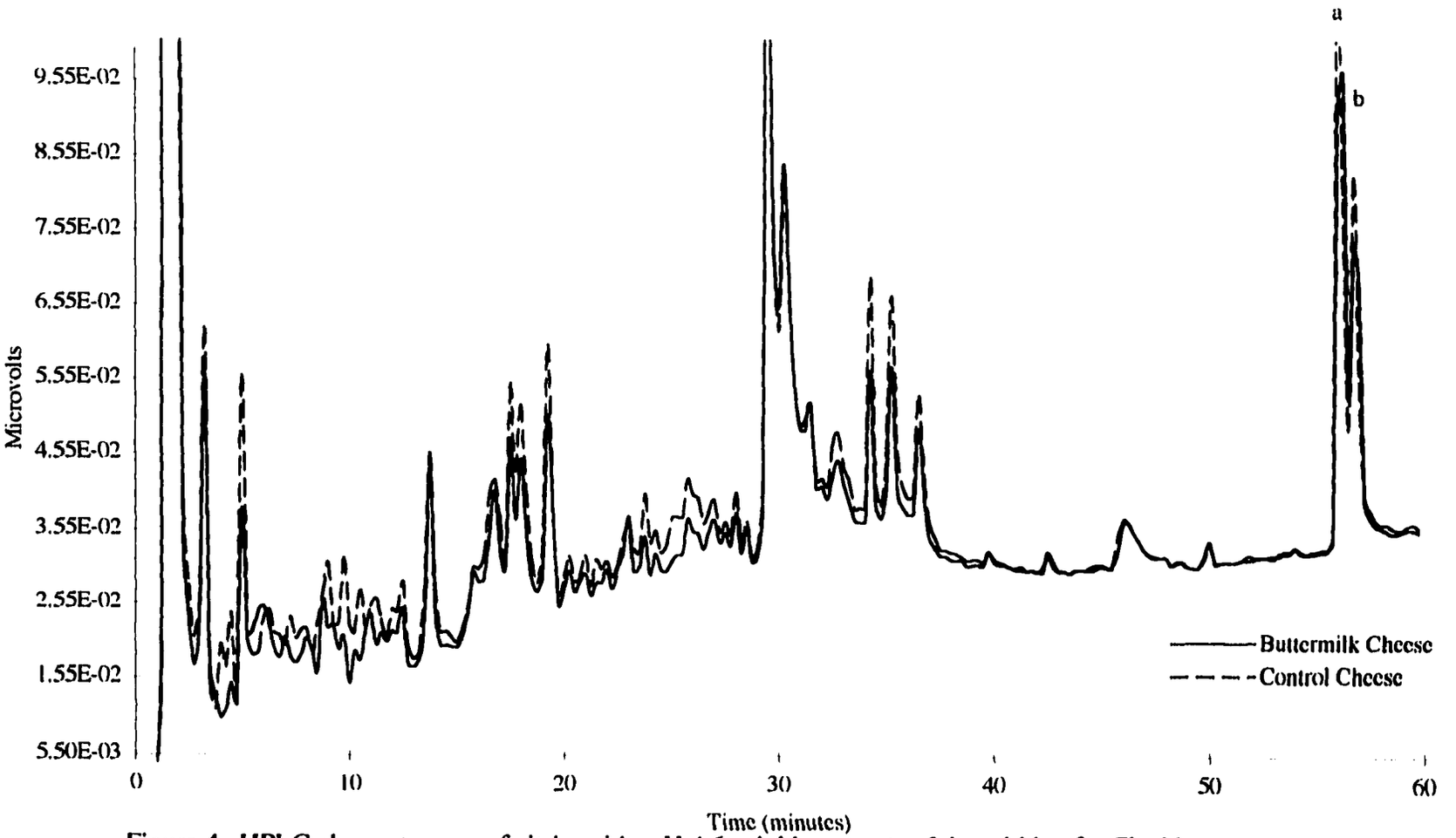

Figure 4. HPLC chromatogram of citric acid - pH 4.6 soluble extracts of day old lowfat Cheddar cheese. Letters indicate peaks that are greatly reduced after one month of aging.

Table 2. Mean peak area for reversed phase HPLC chromatograms of day old and month old cheeses peaks "a" and "b".

Treatment[1]	Peak a		Peak b	
	Day 1	Month 1	Day 1	Month 1
Control Cheese	1281000a[2]	34000b	4600000a	290000b
Buttermilk Cheese	750000a	40000b	2600000a	250000b

1. Treatments: Control Cheese is combined peak areas for homogenized cream lowfat Cheddar. Buttermilk Cheese is combined peak areas for homogenized cream lowfat Cheddar with 1% buttermilk added.
2. Means with different letters indicate a significant difference ($p < 0.05$).

Table 3. Mean peak area for reversed phase HPLC chromatograms of four month old cheeses peak "a".

Treatment[1]	Peak a
Control	1900000
Buttermilk	2500000

1. Treatments: Control Cheese is combined peak areas for homogenized cream lowfat Cheddar. Buttermilk Cheese is combined peak areas for homogenized cream lowfat Cheddar with 1% buttermilk added.

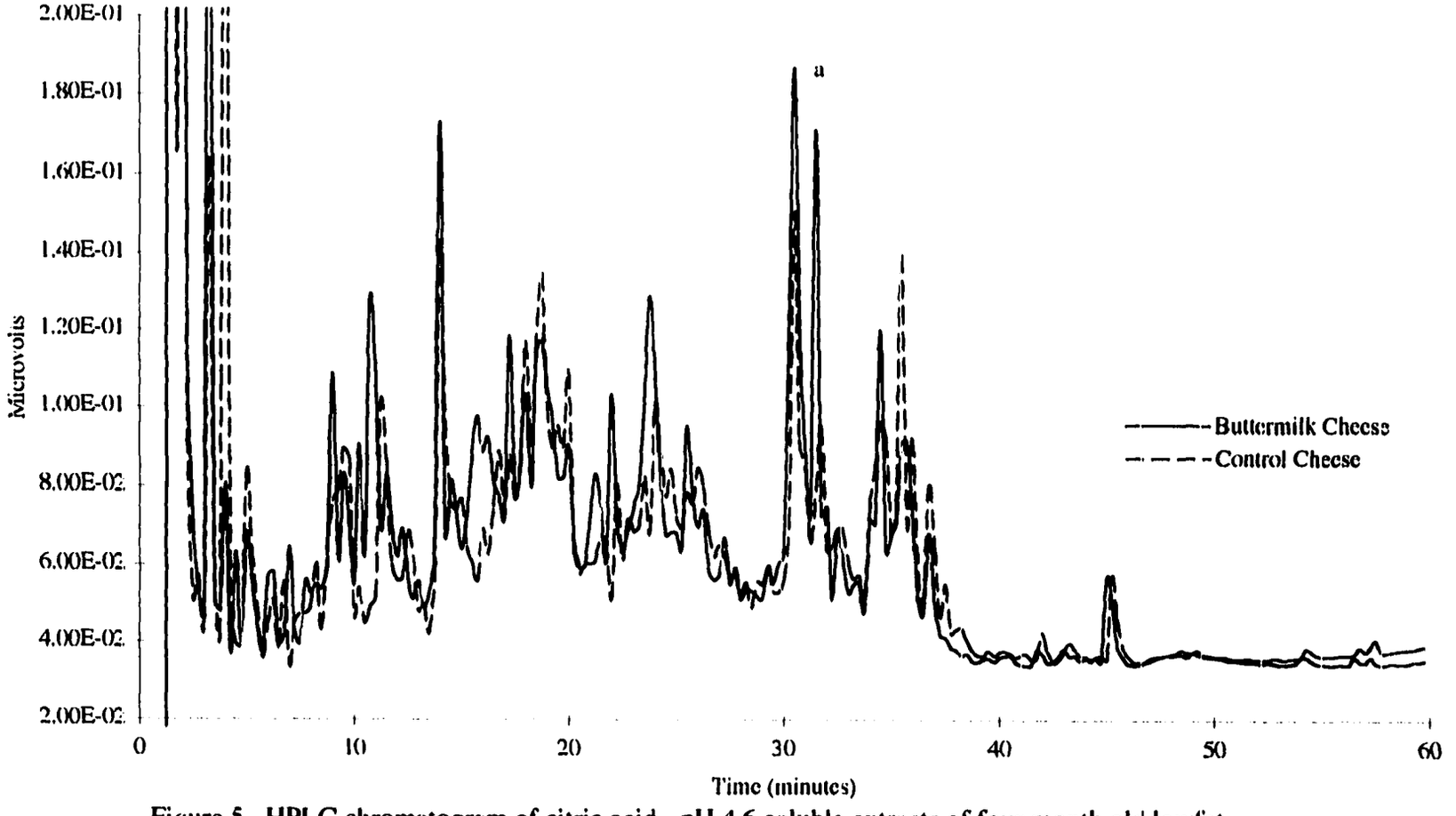

Figure 5. HPLC chromatogram of citric acid - pH 4.6 soluble extracts of four month old lowfat Cheddar cheese. Letters indicate peaks that differed consistently between buttermilk and control cheeses.

Table 4. Mean concentration in parts per million (μg/ml) of free fatty acids in lowfat Cheddar cheese.

Time[1]	C_2				C_4				C_6				C_8			
	CN[2]	CW	BN	BW	CN	CW	BN	BW	CN	CW	BN	BW	CN	CW	BN	BW
1 D	3344	6175	2839	3017	94.1	80.9	78.9	78.9	3.3	3.1	4.2	3.3	0	0	0.7	0.6
1 M	7982	6972	5823	3769	100.4	90.2	82.7	87.0	5.5	5.7	6.0	5.5	0.3	0.5	0.9	1.2
2 M	7610	7231	6500	5691	93.0	97.3	84.7	79.2	4.9	12.0	4.8	4.2	0	0.5	0	0
4 M	2775	2713	2508	2466	66.4	63.4	68.0	69.2	5.7	5.4	5.1	6.5	0.9	0	0.4	0
Time	C_{10}				C_{12}				C_{14}				C_{16}			
	CN	CW	BN	BW	CN	CW	BN	BW	CN	CW	BN	BW	CN	CW	BN	BW
1 D	10.3	4.4	21.8	8.4	8.7	10.4	10.8	4.4	52.7	22.6	38.4	34.0	133.5	107.5	110.8	119.6
1 M	4.2	5.0	3.8	2.5	11.1	10.7	12.7	10.5	25.0	32.2	24.4	33.6	147.5	142.0	121.5	160
2 M	9.5	5.2	4.5	3.0	9.2	9.3	9.1	9.8	57.6	25.1	26.4	13.8	110.4	115.6	93.3	79.6
4 M	6.7	6.4	2.7	5.2	10.7	10.6	6.2	7.8	10.8	16.3	17.4	17.0	105.0	117.3	113.5	114.2

1. Time = sampling at day one (1 D), one month (1 M), two months (2 M), and four months (4 M) of aging.
2. Treatments are control homogenized cream lowfat Cheddar (C) buttermilk homogenized cream lowfat Cheddar with 1% buttermilk powder added (B). Normal curd is the normal milling to salting procedure (N). Washed curd is the washing of the milled curds prior to salting (W).

(table continued)

Time	C_{18}				$C_{18:1}$			
	CN	CW	BN	BW	CN	CW	BN	BW
1 D	317.8	193.3	154.2	152.5	159.0	158.7	198.0	190.2
1 M	189.7	172.5	219.7	200.1	261.4	285.6	301.5	291.2
2 M	337.6	252.7	185.3	147.6	203.4	204.5	194.7	174.2
4 M	165.1	142.1	153.4	157.5	268.1	241.2	240.7	238.3

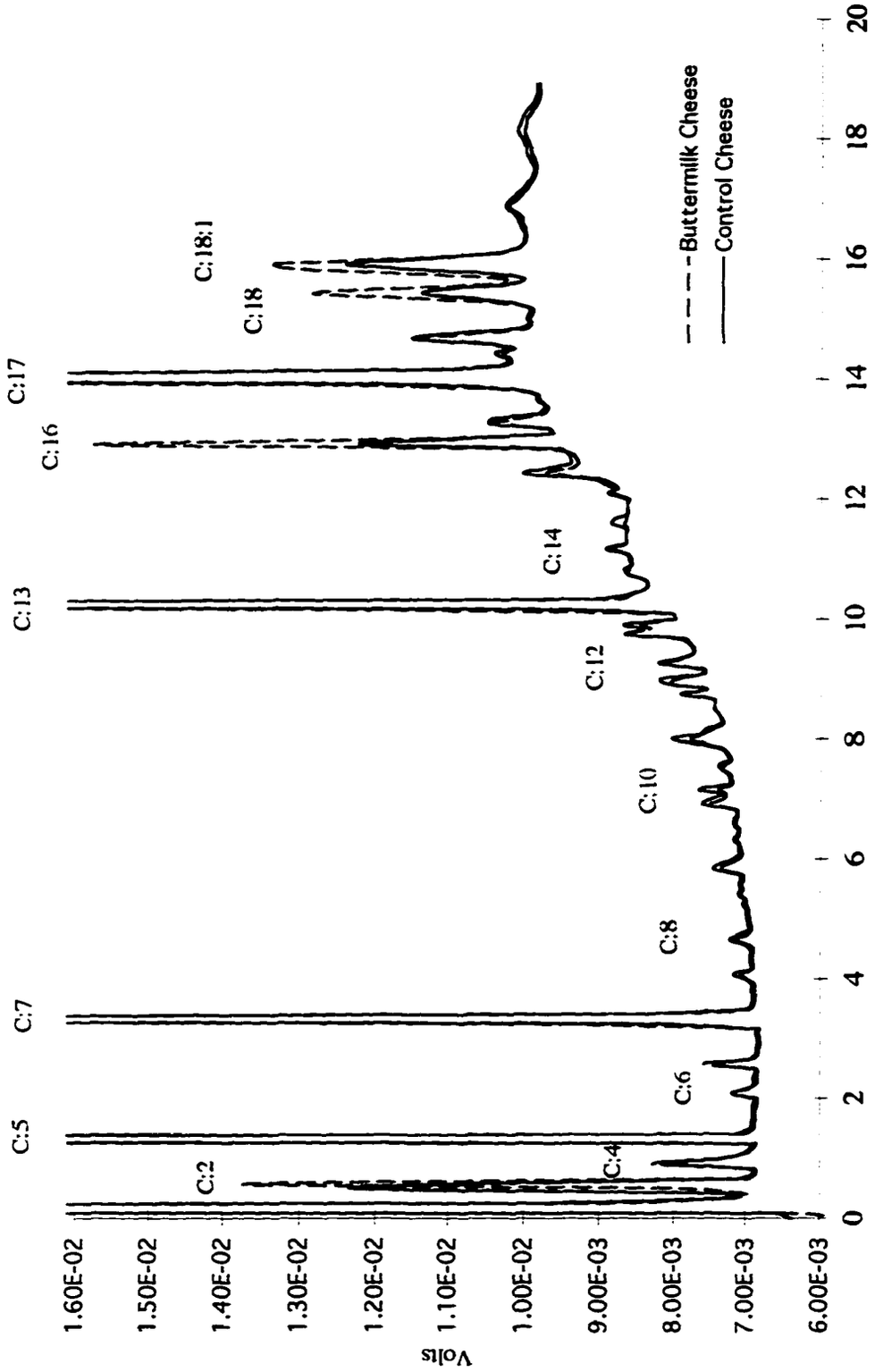

Figure 6. Gas chromatogram of free fatty acids in lowfat buttermilk and control Cheddar cheese.

There was no difference in the levels of butyric acid and the mean values were lower for the buttermilk cheeses at all times except at four months of aging. Ardö (1997) stated that cheeses with lower fat contents had lower concentrations of C_4, C_6, C_8 but not C_{10}, C_{12}, C_{14} or C_{16} when compared to their full fat counterparts. De Jong and Badings (1990), using the same method of extraction in this case, published their results of a full fat Cheddar cheese aged for one year. Comparing De Jong and Badings (1990) results with full fat cheese to the lowfat cheeses in this study, the levels of C_2 and C_4 were greater in the lowfat cheese than the full fat cheese. Woo and Lindsay (1982) reported both lower and higher levels of C_4 than were quantified in the lowfat cheese. These researchers quantified the free fatty acids at one and 10 months in three different Cheddar cheeses. The 10 month samples had a greater quantity of all of the fatty acids than the one month samples. Woo and Lindsay (1982) noted rancid flavors were detected in the cheeses that contained greater than 308 ppm. All lowfat cheeses in this study contained less than that. No data was reported for the time between, however, so the trends could not be compared. Butyric acid (C_4) is generally not desirable at high levels as it is associated with rancid flavors although fatty acids ranging from C_4 to C_{10} have been implicated as contributors to rancid flavors (Weihrauch, 1997). The remaining fatty acids were reduced in the lowfat cheese. Even though buttermilk powder has the possibility of adding greater proportions of triacylglycerols containing stearate (C_{18}) and palmitate (C_{16}) associated with membrane proteins, this effect was not noted in the buttermilk cheeses. The control cheeses generally had higher mean concentrations, although not significant, for most of the fatty acids with the exception of capric (C_{10}) and oleic acid ($C_{18:1}$) in day old cheese. Free fatty acid concentrations, except at levels where rancidity is noted, are not thought to be important contributors to Cheddar flavor (Aston and Dulley, 1982; Woo and Lindsay, 1982).

Microbiology

No significant differences were seen between the treatments for the mean log number of lactic acid bacteria enumerated. There was a significant linear increase in mean log numbers enumerated over time for all treatments (Figure 7). The numerical means of the buttermilk treatments were always greater than those for the control cheeses. The difference at month two appears to show a difference but it is not significant at the 0.05 level.

Texture

There was no significant difference between buttermilk and control cheeses for the load applied at 30% compression. The curd treatment and time of aging were significant. The washed curd treatment initially had the greater load at 30 % compression but this was reversed after month one for the buttermilk cheese and at month two for the control cheese (Table 5). The load applied by the buttermilk cheese increased during the aging period while the load for the control cheese decreased. It was not possible to measure the peak force of the sample so a measure of firmness could not be achieved. The mean values at 30 % compression were less for the control cheese than for the buttermilk cheeses. This may be a result of the structure that was noted in the control cheeses. Informal personal sensory observations by the researcher and colleagues indicated that the buttermilk cheese had a softer, smoother texture. The control cheese had a more rubbery, springy texture and the texture had a stringy grain to it like mozzarella cheese. This was especially evident during the first two months. The Instron data does not seem to reflect these sensory observations. One of the difficulties with compression data is to know which portion of the curve to measure. The most commonly measured point is the maximum force the sample takes before it breaks down. This is commonly used as a measure of firmness, presumably simulating the first bite during mastication with greater force at the maximum indicating greater

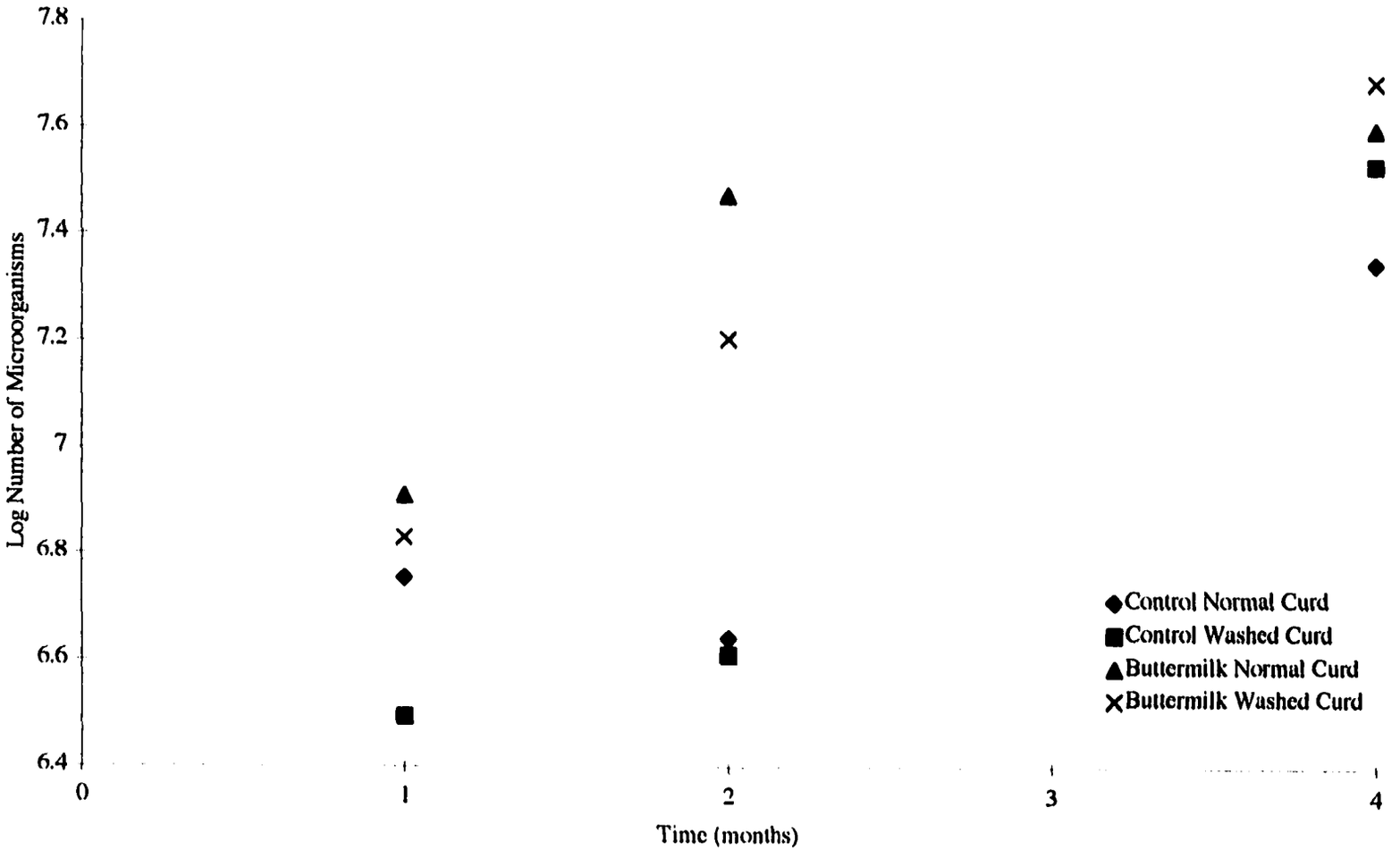

Figure 7. Mean log number of miroorganisms enumerated on Lactobacilli MRS agar in lowfat Cheddar cheese aged at 6°C.

Table 5. Mean load applied at 30% compression of homogenized cream lowfat Cheddar cheese on an Instron Universal Testing machine.

Time	Control Normal Curd[2]	Control Washed Curd	Buttermilk Normal Curd	Buttermilk Washed Curd
Day 1	0.509[1]	0.586	0.567	0.582
Month 1	0.374	0.451	0.642	0.559
Month 2	0.453	0.448	0.821	0.604
Month 4	0.545	0.415	0.850	0.719

1. Load is in kg at 30% compression of 1 cm cubes of cheese with a crosshead speed of 2 mm/min.
2. Treatment = Control is homogenized cream lowfat Cheddar. Buttermilk is homogenized cream lowfat Cheddar with 1% buttermilk powder added. Normal curd is the normal milling to salting procedure. Washed curd is the washing of the milled curds prior to salting.
3. n = 12 for each treatment / time.

firmness (Prentice, 1995). No break point was observable in this case due to limitations of the load cell. The load cell could not receive a load greater than 1 kg and no break point occurred before that point. The mean load values were always greater for the buttermilk cheeses than for the control cheeses at 30 % compression. The washed curd treatments were significantly different from the controls. Measuring at day one, the washed curd treatments applied a greater force to the load cell. For the control, the force of the washed curd treatment was also greater at month one but less at months two and four. In the buttermilk cheeses, the washed curd treatments applied less force to the load cell from month one onward. Possibly this stringy grain to the protein in the control cheeses indicates a point of weakness where cracks may develop and the cheese structure breaks down when being compressed. This stringiness could also cause a difference in the relaxation processes applied to the load cell by the cheese as described by Shama and Sherman (1973).

Conclusions

Adding buttermilk powder to a level of 1 % of the weight of the cheese milk in a 72 % reduced fat cheese changed some of the chemical properties of the resulting Cheddar cheese. Buttermilk powder addition increased the moisture content, and MNFS. This increase in MNFS would be expected to produce a cheese with a softer texture.

The mean force applied to the load cell at 30% compression was significantly different for the curd treatments with the Instron compression analysis. The time of aging was also significantly different. Membrane proteins from the buttermilk powder that stained with Coomassie Brilliant Blue were mainly lost to the whey. One peak in the HPLC chromatograms at four months was present at consistently greater levels in the buttermilk cheeses than in the control. It was generated during aging and may be due to increased enzyme activity on a specific protein. This could possibly be a factor in

the increased sulfide flavor that was noted in informal sensory evaluation of the buttermilk cheeses. Kristoffersen (1985) reported that little attention has been given to carbohydrate metabolism and the metabolism of carbon compounds that would involve oxidation - reduction phenomena. He noted that oxidation-reduction reactions are important for flavor and are probably the direct result of microbial metabolism. Kristoffersen further noted that sulfhydryl groups were formed as a result of oxidation-reduction reactions. The higher moisture levels and the possibility of greater lactose levels in the buttermilk cheeses could be responsible for flavor differences.

CHAPTER 3: SENSORY EVALUATION OF LOWFAT CHEDDAR CHEESE

Introduction

Consumer and trained taste panels form the basis for any food product development decisions. Any process or ingredient change in a product must be correlated with sensory data since the finished product is meant to be consumed by the public. In this study, buttermilk powder was added to lowfat Cheddar cheese to determine if this could improve texture and flavor properties. Chemical properties of the cheese with buttermilk were different, justifying evaluation of the cheese organoleptically. One objective of the consumer panel experiment was to determine if consumers could differentiate between cheeses with and without added buttermilk powder. A second objective was to evaluate if consumers preferred the properties of one cheese over the other. A third goal of the study was to determine if specific flavor and texture attributes changed over time. For this purpose, a panel of experienced evaluators was assembled to evaluate resultant cheeses by descriptive analysis.

Materials and Methods

Cheese Manufacture

High temperature short time pasteurized skim milk was purchased from a local dairy plant in 5 gallon bags and was weighed into two vats in the amount necessary to standardize the cheesemilk to 0.9 % fat after homogenized cream addition. The amount of pasteurized cream required to standardize the skim milk in each vat, plus skim for diluting the cream for homogenization was weighed into two cans. The amount of skim milk added for diluting was two times the weight of the cream. For the buttermilk cheese, the buttermilk powder was calculated as containing 4 % fat and this was worked

into the standardization equation. Extra grade spray process dry sweet cream buttermilk powder (California Milk Producers, Artesia, CA) was added (1% on a weight basis) to the proper amount of diluted cream and mixed well. Both the cream containing buttermilk powder and the control were homogenized at 15.8 MPa on a two-stage homogenizer and added to the vats. Milk in the vats was brought to 32.3°C and 1% Redi-set RFC 300 culture was added (Chr. Hansens's Laboratory, Inc., Milwaukee, WI). The cheesemilk was allowed to ripen for 45 min. Calcium chloride was added at a rate of 0.02 % (by weight). Milk was set with Chy-Max II fermentation produced chymosin (Pfizer, Inc., Milwaukee, WI) for 30 min. Curd was cut with 0.95 cm knives, allowed to rest for 5 min and then brought to 37.8°C over 30 min with gentle stirring. After draining, trenching and knitting, curd was cheddared for one h and then milled. The curd was then salted at a rate of 1.27 kg salt per 453.6 kg cheesemilk. The cheese was hooped in 20 lb Wilson hoops and pressed overnight at 275.8 kPa.

Consumer Panel

Three consumer panels were assembled at two, three and four months of cheese aging. The panels were held on the Louisiana State University campus. Each panel was advertised by posting flyers in campus buildings and by posting a bright sign near a large lecture hall advertising free ice cream for tasting cheese. The panelists were informed, before entering the sensory room, how much time was required and how to fill out the form and evaluate the cheese. All panels were conducted in a sensory room containing ten individual table-top partitioned booths under fluorescent light. Panelists were provided with a pencil, evaluation form, water, two crackers, a napkin and toothpicks. They were instructed to taste and swallow the samples and to use crackers and water to freshen their palate if desired. A room monitor was present to answer any questions , prepare booths between panelists, receive finished evaluation forms, and to distribute a cup of LSU ice cream as compensation for participating in the panel.

Panelists were directed to a prepared booth upon entering the tasting room. The evaluation form consisted of two pages of demographic questions, a simple difference test of flavor, a simple difference test of texture, and two preference test forms. The procedure was to first fill out demographic and cheese consumption information and then sample the products. Six samples of cheese, approximately 2 cm cubes at 5°C were placed in 60 ml, capped soufflé cups on a styrofoam plate. Each cup had a three digit randomly generated code written on it. A key was generated for up to 60 panelists in order to randomize treatments. The panelist number was written on the plate and form and the correct samples were placed on the plate.

Two codes were generated for the buttermilk cheeses and two for the normal cheeses for the flavor difference test. Two different codes were used for each of the cheeses for the texture difference test. Panelists received either a placebo of two buttermilk or two control cheeses or they received one of each cheese in random order. This procedure meant that each panelist received one out of a total of four possible combinations for both the flavor and texture difference tests. Panelists were requested to taste the two samples of cheese indicated on the first page of their form and evaluate them for flavor. The panelists had been instructed that the two samples could be the same cheese sample or that they could be different. The panelist indicated, based on flavor, if they thought the samples were the same or different. If they thought they were different samples, they were requested to indicate, by number, which sample had the stronger or more intense flavor. The panelist then tasted the next two samples indicated on their form, which was again randomly assigned from the four combinations , and evaluated them for texture by the same simple difference test. If they thought the samples were different, they were asked to indicate, by number, with one had the softer texture. The final two samples were evaluated for flavor, texture and overall preference ratings. The final samples were one buttermilk cheese and one control, presented in

random order to the panelists. The panelists rated the samples on a nine point scale, one being "Dislike Extremely", nine being "Like Extremely". Panelists were also asked if the product was acceptable to them and if they would purchase it if commercially available. They answered yes or no to these questions.

Experienced panel

The experienced panel consisted of 8 panelists skilled at evaluating Cheddar cheese for common attributes by the American Dairy Science Association (ADSA) (Bodyfelt *et al.*, 1988) scoring method. One was a professor and coach of the LSU dairy product evaluation team. Three undergraduate and two graduate students, all members of past ADSA judging teams also participated. The final two panelists were a less experienced but very motivated staff member and graduate student in the dairy science department. A 15 cm scale was used to score the intensity of each attribute. At the initial training period, panelists compared the experimental cheeses to commercial varieties and practiced with the 15 cm scale and the ADSA descriptors. It was determined that additional descriptors were necessary, while others were found unnecessary to describe the product attributes. "Springy" was defined as the degree the cheese cube recovered from being squeezed lightly between the fingers. "Stringy" was defined as being the degree the cube would string like mozzarella when it was pulled apart. "Flavor Intensity" was the overall strength of the combination of flavors. "Aroma Intensity" was the overall strength of the aroma when smelled from the hand at breakdown. Kraft Cracker Barrel Sharp cheese was described as being about three fourths of the way along the scale (about 11.25 cm) in intensity for both flavor and aroma. Samples were evaluated 6 times throughout aging. Two cubes of about 2 cm cubed of both the buttermilk and control cheese were presented on styrofoam plates. The plate was divided into two sectors by a pen line and a three digit code was written on each half with the corresponding cubes placed above the number. Panelists were

instructed to taste the samples from left to right. Half the panelists received the buttermilk sample first and the other half received the control sample first. The samples were at about 10°C. Samples were tasted under fluorescent lights at a conference table without partitions. Panelists worked individually and no discussion took place. Reference samples of the cheeses were placed on plates in the center of the table to evaluate the "gassy" and "open" scores. Panelists next evaluated "springy" and then "stringy" before breaking the sample down in their hand for the remaining texture measures. Lastly, the flavor attributes were evaluated.

Statistical Analysis

The simple difference tests of the consumer panel were evaluated, within each consumer panel, by chi-squared analysis using a Power Macintosh 6100 computer and Microsoft Excel version 5.0a software for the Power Macintosh (Microsoft Corporation, Seattle, WA). The chi-squared statistic was generated by hand on a spreadsheet and compared to the critical value at p=0.05 from a table (Meilgaard *et al.*, 1987). The consumer preference tests were evaluated within each panel for flavor, texture, and overall acceptability by a two sided paired t-test. The expert panel descriptive analysis was evaluated by the General Linear Model Procedure of SAS for Windows ver. 6.11 (SAS, Cary, NC). The model statement was Treatment Panelist(Treatment) Time Time*Treatment. Treatment being buttermilk cheese or control cheese while time was the weeks of aging. The Panelist(Treatment) mean square was used as the error for the Treatment effect.

Results

Consumer panel results

A total of 162 consumers participated in the three panels. Demographic results are listed in Table 6. As can be seen from the table, the panel was biased educationally in that all of the panelists had achieved their high school degree. All but one were

Table 6. Consumers responses to demographic information questions.

Mean Age of Panelists	28
Race	
White	75 %
Asian	10 %
Black	5 %
Spanish/Hispanic	5 %
Other	5%
Education Achieved	
High School	1 %
Attending College (first degree)	43 %
Complete Undergraduate Degree	19 %
Complete Graduate or Professional Degree	38 %
Employment Status	
Full-time	24 %
Part-time	17 %
Unemployed	2 %
Student	57 %
Mean Household Income Before Taxes	$ 26,300
Cheese Consumed on a Weekly Basis	
Cheddar	69 %
Mozzarella	44 %
Swiss	24 %
Monterey Jack	17 %
Latin American	2 %
American/Process Cheese	39 %
Cream Cheese	25 %
Other	15 %
Type of Cheddar Purchased	
Mild	22 %
Medium	38 %
Sharp	18 %
Extra Sharp	9 %
Do Not Purchase Cheddar	13 %
Frequency of Reduced Fat Cheese Product Purchases	
Never	23 %
Rarely	38 %
Sometimes	18 %
Often	16 %
Only Purchase Reduced Fat Cheese Products	5 %
Most Important Factor in Cheese Purchase Decision	
Price	23 %
Brand Name	2 %
Taste	63 %
Nutritional Attributes of the Product	11 %

attending or had completed undergraduate and 38 % had completed a graduate or professional degree. In the panel evaluating the cheese at 2 months, there was a significant difference between the texture of the cheeses in both the difference test and the preference test (Table 7). Results for consumers correctly identifying the samples as different indicated that consumers found the buttermilk cheese as being softer and the control cheeses as having the more intense flavor (Table 8). Means for flavor, texture and overall liking on the preference test were higher for the buttermilk cheese than for the control cheese. There was a significant difference in the consumer preference for flavor and overall liking in the third and fourth month (Table 7). The control cheese scored significantly higher than the buttermilk cheese in the third and fourth month. Means for texture were higher but not significant for the control cheese. Consumers in the 2nd and 3rd panels were unable to significantly differentiate the cheeses for flavor or texture by the simple difference test. Of those consumers who correctly identified the samples as different in the third and fourth months, the majority found the control cheeses to have the softer texture (Table 8). In the third month the consumers found the control cheese to have the more intense flavor but in the fourth month they found the buttermilk cheese to have the more intense flavor. Results of the "yes"/ "no" questions of acceptability and intent to purchase varied similarly (Table 9). The first panel, evaluating 2 month old cheese, found the buttermilk cheese to be more acceptable. A greater percentage of panelists stated that they would purchase the 2 month old buttermilk cheese. The second and third panels found the 3 and 4 month old control cheeses to be more acceptable and purchasable. A significant difference was found for cheeses aged 4 months. The number of consumers who stated they would purchase the 4 month old control cheese was significantly higher than the number who stated that they would purchase the buttermilk cheese.

Table 7. Consumer mean preference scores for flavor, texture, and overall acceptability.

Treatment[1]	2 Months Aged n = 46			3 Months Aged n = 59			4 Months Aged n = 57		
	Flavor	Texture	Overall	Flavor	Texture	Overall	Flavor	Texture	Overall
Buttermilk Cheese	6.6	6.7a[2]	6.5	6.0a	6.2	5.8a	6.3a	6.7	6.3a
Control Cheese	6.4	6.2b	6.3	6.8b	6.6	6.7b	6.9b	6.8	6.9b

1. Treatment = Control cheese is homogenized cream lowfat Cheddar. Buttermilk cheese is homogenized cream lowfat Cheddar with 1% buttermilk addition.
2. Means with different letters indicate a significant difference ($p < 0.05$).

Table 8. Consumer results of flavor intensity and texture questions.

Treatment[1]	2 Months Aged		3 Months Aged		4 Months Aged	
	More Intense Flavor n = 15[2]	Softer Texture n = 17	More Intense Flavor n = 20	Softer Texture n = 11	More Intense Flavor n = 19	Softer Texture n = 13
Buttermilk Cheese	33 %	82 %	15 %	36 %	68 %	38 %
Control Cheese	66 %	18 %	85 %	64 %	32 %	62 %

1. Treatment = Control cheese is homogenized cream lowfat Cheddar. Buttermilk cheese is homogenized cream lowfat Cheddar with 1% buttermilk addition.
2. n = indicates the number of people who correctly responded to the difference question.

Table 9. Percent of consumers finding samples acceptable and stated purchase intent.

Treatment[1]	2 Months Aged n = 48		3 Months Aged n = 59		4 Months Aged n = 57	
	Accept.	Purchase Intent	Accept.	Purchase Intent	Accept.	Purchase Intent
Buttermilk Cheese	91 %	87 %	79 %	72 %	86 %	68 %a[2]
Control Cheese	85 %	76 %	90 %	83 %	91 %	84 %b

1. Treatment = Control cheese is homogenized cream lowfat Cheddar. Buttermilk cheese is homogenized cream lowfat Cheddar with 1% buttermilk addition.
2. Percents with different letters indicate a significant difference ($p < 0.05$).

Experienced panel results

Mean values for the control cheese for openness, springiness, firmness, crumbliness and curdiness were greater than for the buttermilk cheese over most of the study (Table 10). Values for firmness seemed to approach each other more closely as ripening time increased, and the buttermilk cheese was found to have the greater mean firmness during the final taste panel. Mean scores for bitterness, acid, sulfide, unclean and overall flavor intensity were greater for the buttermilk cheese than for the control throughout most of the test period. None of the differences in the means were statistically significant. The time factor was significant for openness, springiness, stringiness, curdy, bitter, acid, unclean, and overall flavor intensity. Mean values for openness, springiness, and stringiness, bitter, acid, unclean and overall flavor intensity do not appear to be following any trend with time, however, and could be a function of variations in the sample or in scoring by the panelists. Values for the first panel were elevated mainly due to the panelists inexperience with the scoring system. Firmness means appear to be showing a time trend although it was not significant. Curdiness in the control and buttermilk cheese appeared to decrease with time, although the results were, again, variable.

Discussion

Some of the main criticisms of lowfat Cheddar cheese result from a lack of flavor and a firm texture. The cheese produced in this study with added buttermilk powder did produce a cheese with more flavor as evidenced by the means of the trained panel. The cheese had an overall more intense flavor and greater acid and sulfide flavor which is desirable in a sharp Cheddar cheese product. Buttermilk cheese had more bitterness, but the mean scores were very low on the intensity scale and would probably not influence consumers. Cheese containing buttermilk had a softer texture, at least initially, which is a desirable improvement. The consumer panel determined this to be

Table 10. Mean values of experienced panel for attributes in homogenized cream lowfat Cheddar cheese.

Weeks Aged	Gassy		Open		Springy		Stringy		Firm		Crumbly	
	B	C	B	C	B	C	B	C	B	C	B	C
6	3.6	3.5	6.5	5.4	9.1	10.1	7.3	5.7	7.1	7.8	3.0	4.4
8	3.7	2.4	2.3	4.6	6.4	6.3	5.0	4.3	6.9	7.5	2.7	5.3
11	4.3	1.7	1.9	4.2	4.8	8.1	3.9	5.3	5.9	6.9	1.7	3.8
13	3.4	4.2	2.8	4.9	5.9	6.0	5.3	5.1	5.6	6.4	2.2	3.9
15	4.1	3.3	2.8	4.7	3.5	5.5	3.4	4.4	5.7	5.8	2.5	3.6
17	2.8	3.8	3.6	4.6	3.9	4.3	2.5	3.9	4.4	3.9	1.5	2.1

1. Treatment = Control cheese is homogenized cream lowfat Cheddar (C). Buttermilk cheese is homogenized cream lowfat Cheddar with 1% buttermilk addition (B).

(table continued)

Weeks Aged	Curdy		Mealy		Bitter		Acid		Sulfide		Unclean	
	B	C	B	C	B	C	B	C	B	C	B	C
6	3.7	5.4	2.3	3.9	3.4	2.6	6.1	6.2	2.3	3.4	5.4	2.2
8	4.2	3.5	2.1	3.0	1.5	1.0	5.1	3.5	2.1	1.8	1.6	1.1
11	2.5	3.3	1.8	2.1	2.6	1.4	4.0	3.0	2.6	2.4	2.5	0.9
13	3.0	4.0	1.5	1.4	2.4	1.0	4.6	3.3	3.5	2.3	2.8	0.8
15	2.4	2.9	1.8	1.9	4.3	3.2	6.2	3.5	3.5	3.4	1.8	2.3
17	1.8	2.6	2.0	1.1	2.6	2.9	5.2	3.3	4.4	3.8	2.5	2.0

(table continued)

Weeks Aged	Flavor Intensity		Aroma Intensity	
	B	C	B	C
6	6.5	7.1	3.9	4.9
8	4.2	3.0	2.9	1.8
11	5.0	3.5	3.9	2.2
13	6.3	3.7	3.5	3.3
15	6.0	5.9	3.2	4.8
17	5.7	4.8	4.2	3.5

the case in the first panel when the cheese was aged 2 months. Experienced panelists also indicated this difference in texture by their higher scores for firmness and curdiness in the control cheese. Consumer panelists did not like the buttermilk cheese as aging time increased. This correlated with the increase in acid and sulfide flavor noted by the experienced panel. The preference test for flavor was the first one on the scorecard. It is likely that this score also affected the scores consumers gave in the other categories of the preference test. Any benefit in the texture of the buttermilk cheese was probably outweighed by the dislike of a more aged flavor. A larger proportion of the panelists answered that they purchase mild and medium Cheddar cheese rather than sharp or extra sharp. Thirteen percent of the panelists did not purchase Cheddar cheese at all. These facts might explain why the panel preferred the milder, control cheese in the 2nd and 3rd taste panels. Consumers filled out demographic forms themselves so it is also possible that panelists confused "Cheddar Cheese" with "American Cheese" when reporting what type of Cheddar cheese they purchased.

CHAPTER 4: CONCLUSIONS

The addition of buttermilk powder to lowfat Cheddar cheese by homogenizing it into the cream portion of the cheesemilk changed the properties of the cheese in this study. Previous research has shown that homogenizing only the cream portion of the cheesemilk can improve the texture and water binding of lowfat cheese without adversely affecting coagulation (Metzger and Mistry, 1994). The addition of buttermilk powder in this study improved the moisture binding, texture and increased flavor intensity.

The overall results can be summarized as follows:

1. The addition of buttermilk powder softens the cheese and therefore improves the texture during the early phase of ripening.

2. Homogenizing the cream produced 71% reduced fat cheeses that the majority of consumers in this study found acceptable and stated they would purchase them if commercially available.

3. The mean scores of the experienced panel for bitterness, acid, sulfide, unclean and overall flavor intensity were greater for the buttermilk cheese than for the control throughout the aging period.

4. Consumer panelists preferred the less intense flavored control cheeses after three months of aging. The lack of off-flavors and the presence of clean, mild flavors are probably the most important characteristics for consumers when evaluating small sampies of natural cheese for preference.

Organoleptic evaluation is the most important feature to any food product development initiative. One study showed that "Taste" and "Family doesn't like it" were the two biggest barriers to lowfat cheese consumption (Barr, 1990). If the consumers

do not like the flavor, they most likely will not purchase the product. It is important, therefore, to correlate flavor changes with physical measurements. In this study, it was attempted to compare physical measurements of moisture, protein, number of microorganisms, proteolysis, free fatty acids, and texture with consumer and experienced panels. Significant results for the main effects in the production and sensory chapters are summarized in Tables 11 and 12. It can be seen in the table that the addition of buttermilk to the cheesemilk caused significant differences in the percent moisture and in the moisture in non-fat substance. The curd treatments, washed or not, were significantly different for percent moisture and by the Instron texture analysis.

The consumer panelists in this study noted that the buttermilk cheese had a softer texture at two months than the control cheese. The experienced panel also considered the buttermilk cheese to be softer initially but the difference between the two cheeses decreased over time. The results of the Instron texture analysis were inconclusive. It was not possible to measure the peak force of the sample so a measure of firmness could not be achieved. The mean values at 30 % compression were less for the control cheese than for the buttermilk cheeses which would seem to indicate the control cheese had the softer texture. This may be a result of the structure that was noted in the control cheeses causing points of weakness during compression. The most apparent reason for the softer texture initially is the greater moisture in non-fat substance content of the buttermilk cheeses. During aging, proteolysis will expose more polar residues and allow them to bind more moisture. Cheddar cheese has been noted to become harder with age for this reason (Lawrence *et al.*, 1987). The benefits of increased moisture may then have declined with time. No difference between the cheeses was noted in the amount of soluble nitrogen or the rate at which it was produced in each cheese. If there had been differences in proteolysis, this could have explained the differences in texture.

Table 11. Statistically significant results for cheese composition data with source and probability.

Moisture		
WP[2]		0.0001[1]
SP		0.0001
Protein (wet basis)		
SP		0.0042
Moisture in Non-fat Substance		
WP		0.0135
Microorganisms		
Time		0.0001
Citric Acid - pH 4.6 Soluble Extracts		
Time		0.0001
Texture Analysis		
SP		0.0182
Time		0.0004
Free Fatty Acids		
C_2	Time	0.0001
C_4	Time	0.0001
C_{10}	Time	0.0253
C_{14}	Time	0.0137
C_{16}	Time	0.0116
C_{18}	Time	0.0374
C_{18}	SP	0.05
$C_{18:1}$	Time	0.0001

1. Probability is $p < F$.
2. WP = buttermilk or control whole plot. SP = normal or washed curd treatment subplot. Time = sampling times during aging.

Table 12. Statistically significant results for sensory data with source and probability.

Consumer Panel Simple Difference Test		
Panel 1	Texture	0.001[1]
Consumer Preferences		
Panel 1	Texture	0.05[2]
Panel 2	Flavor	0.00034
Panel 2	Overall	0.00257
Panel 3	Flavor	0.00452
Panel 3	Overall	0.01146
Experienced Panel Results		
Open	Time	0.0248[3]
Springy	Time	0.0001
Stringy	Time	0.0135
Curdy	Time	0.0251
Bitter	Time	0.0175
Acid	Time	0.0139
Flavor	Time	0.0038

1. Probability for difference test is $p < x^2$.
2. Probability of consumer preference test is $p < t$ (two tailed).
3. Probability for experienced panel is $p < F$.

The greater mean log number of lactic acid bacteria could be explained by the greater moisture content and the additional lactose that the buttermilk powder contained. Most lactose is lost to the whey, but a brown color observed in the buttermilk cheese during moisture analysis by drying seems to indicate that the buttermilk cheeses retained more lactose. This greater mean number of organisms would provide greater levels of enzymes in the cheese after they die and lyse. The number of viable cells in the control cheeses approached levels equal to the buttermilk cheese by the second month but possibly not in the total mass of cells. El Soda (1997) reported a study that indicated more than 85% of the starter cells are located at the peripheral region of the fat globule when viewed by electron microscopy. Differences in the fat/water interface in respects to the starter culture could have contributed to the differences in flavor.

The HPLC and electrophoresis results cast doubt as to whether novel proteins are incorporated into the cheese by the addition of buttermilk powder. Most of the proteins that were particular to the buttermilk powder were lost to the whey fraction. If those proteins were still part of the MFGM before extraction, it is doubtful whether the phospholipids were incorporated into the cheese as well. Enzymes could have been incorporated into the cheese but they would have had too large a molecular weight to have been identified by the acrylamide gels used in this study. A lower concentration of acrylamide would be necessary to separate these larger proteins.

The mean scores of the experienced panel for bitterness, acid, sulfide, unclean and overall flavor intensity were greater for the buttermilk cheese than for the control throughout most of the test period. This corresponded to the informal sensory evaluation during the chemical analysis phase of this study. The greater flavor intensity correlated with lower preference scores by the consumer panel, however. The consumer panel was extremely biased with young, well educated undergraduate and graduate students. Respondents indicated they most often purchased mild and medium

Cheddar cheeses, not sharp or extra sharp. A broader based consumer panel could yield different results.

Little attention has been given to carbohydrate metabolism and the metabolism of carbon compounds that would involve oxidation -reduction phenomena (Kristoffersen, 1985). Oxidation-reduction reactions are important for flavor and Kristoffersen (1985) noted that sulfhydryl groups were one of the products of these reactions. The higher moisture levels and the possibility of greater lactose levels in the buttermilk cheeses could be responsible for the more intense flavor of the buttermilk cheese, especially in sulfide flavor.

The levels of free fatty acids in the cheese were not significantly different although the means for the control cheeses were generally higher. The buttermilk cheese had a stronger flavor, however. This indicates that either the fatty acids were not important in contributing to the flavor, or that flavors in the buttermilk cheese were more detectable due to changes at the fat/water interface. The fat/water interface is thought to be important for flavor and the differences noted in flavor may be due to the difference in moisture content between the cheeses or due to differences in the membrane at the surface of the fat pockets in the cheese.

The results of this research indicate the need for further analysis. The production of lowfat Cheddar cheese with skim milk powder homogenized into the cream portion and its comparison to buttermilk powder would be a study of interest. This would incorporate about the same amount of lactose and milk proteins into the cheese. Madsen *et al.* (1966) investigated the difference between the addition of fluid and dried skim and buttermilk to cheese. They found that the buttermilk cheeses had more moisture than the skim milk cheeses. This greater moisture was attributed to increased water binding by the buttermilk but also to decreased whey expulsion from the buttermilk cheeses. The buttermilk cheeses in this study had a higher mean score for

unclean flavor by the experienced panelists. This unclean flavor could very well have been whey taint. It would be interesting to investigate the relative amounts of lactose retained in the curds of cheeses made with buttermilk and skim milk powder. Also, any differences in the color of the dried cheese samples would be of interest. Too great a brown color when the cheese is used for cooking might be undesirable.

Buttermilk powder undergoes more severe heat treatments in its commercial production than skim milk powder. Differences in results from the various studies using buttermilk in cheese could reflect the differences in the way the buttermilk is manufactured. The cream used to make the buttermilk in this study was pasteurized at 85 - 88°C for approximately 25 seconds. Skim milk would typically be pasteurized at a temperature of approximately 77°C for 15 - 17 seconds. The cream for making the buttermilk was held for 8 - 36 h before buttermaking. After buttermaking, the buttermilk was stored for 36 - 48 h. The buttermilk was then re-pasteurized at 78°C for approximately 20 seconds before being condensed and dried similarly to skim milk powder. This extra, high temperature pasteurization step and the storage time could lead to differences in the final cheese. This could effect the proteins and lactose and could cause differences in solubility. Plasmin, a natural milk protease is activated by high heat. In light of the long period of storage before being dried, the buttermilk could have had increased incidence of psychrotrophic growth and enzyme activity. Proteolysis could have been taking place in storage although extracts of buttermilk powder showed no appreciable soluble nitrogen and no peaks by HPLC.

Further research could also investigate the role of phospholipids on cheese texture. Differences in incorporation of phospholipids either in pure form or by the addition of buttermilk powder, and the effect of incorporation on texture would be of interest. Many of the new "structured lipid" fat replacers are modified triacylglycerols. Phosphoacylglycerols, like lecithin, are composed of glycerol with two fatty acids and

phosphate bound to another group containing nitrogen. Modification of the fatty acids in phospholipids in a similar way could possibly yield products that could alter the fat/water interface and improve flavor reactions.

Further modifications to steps in the cheesemaking process, and the investigation of other factors such as adjunct culture additions are necessary to improve on the cheeses manufactured in this study. Lowfat cheese research will continue to be a challenge since cheese is a complex mixture and the mechanisms of the reactions involved in its ripening are not yet fully elucidated.

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APPENDIX A: MOISTURE ANALYSIS

Materials

aluminum moisture dishes

balance

tongs

balance

atmospheric drying oven set at 105°C

Methods

1. Approximately 2 g of grated cheese was placed in aluminum drying dishes that had previously been dried and stored in a desiccator and were handled only with tongs.
3. Samples were placed in oven for 16-18 h.
4. Samples were removed and cooled in a desiccator before final weighing.
5. Percent moisture was calculated by :

$$\% \text{ moisture} = (\text{loss in weight/weight of sample}) \times 100$$

APPENDIX B: FAT ANALYSIS - BABCOCK METHOD

Materials

concentrated sulfuric acid

Babcock sulfuric acid

butanol

Babcock bottles for skim milk (18 g), cheese (9 g), and cream (18 g)

bottle shaker

centrifuge, heated to 60 °C.

water bath, heated to 60 °C

food processor

Method for Cheese

1. Shred cheese in food processor.
2. Accurately weigh 9 g of cheese in tared cheese bottle.
3. Pipette 10 ml of 60 °C water into bottle.
4. Mix to thoroughly suspend cheese.
5. Add a total of 15 ml concentrated sulfuric acid in three steps of approximately 5 ml, swirling between additions.
6. Swirl until all lumps are dissolved.
7. Shake on mechanical shaker for 5 min.
8. Centrifuge for 5 min.
9. Add 60°C water to the neck of bottle.

10. Centrifuge 2 min.
11. Add hot water to readable range.
12. Centrifuge 1 minute.
13. Transfer to a 60°C water bath for 5 min.
14. Measure the fat in the calibrated column.

Method for Skim Milk

1. Measure 2 ml normal butanol into 18 g skim milk bottle.
2. Add exactly 9 ml skim milk into test bottle.
3. Add 7-9 ml Babcock sulfuric acid.
4. Shake on mechanical shaker for 5 min.
5. Centrifuge for 5 min.
6. Add 60°C water to neck.
7. Centrifuge for 2 min.
8. Add 60°C water to readable range.
9. Centrifuge for 1 minute.
10. Transfer to 60°C water bath for 5 min.
11. Measure fat in the calibrated neck.
12. Multiply the amount by two since 9 ml were put in an 18 g bottle.

Method for Cream

1. Weigh 9 g of cream into a tared 18g bottle.
2. Pipette 9 ml distilled water and mix.
3. Add a total of 17.5 ml Babcock sulfuric acid in three equal steps, swirling between each addition.

4. Shake on mechanical shaker for 5 min.

5. Centrifuge for 5 min.

6. Add 60°C water to neck.

7. Centrifuge for 2 min.

8. Add 60°C water to readable range.

9. Centrifuge 1 minute.

10. Transfer to 60°C water bath for 5 min.

11. Add two drops of glymol before reading the amount of fat in the column.

12. Multiply the amount of fat by 2 since 9 g were put in an 18 g bottle.

APPENDIX C: KJELDAHL NITROGEN USING THE KJELTEC SYSTEM

Materials

concentrated sulfuric acid

digestion tubes

Kjeltabs

Kjeltec digestion block and distillation unit

Kjeldahl boric acid with color indicator

standardized 0.1M and 1M HCl

40% w/w NaOH

Cheese or citric acid-pH 4.6 soluble nitrogen

Citric Acid-pH 4.6 Soluble Nitrogen Digestion Method

1. Weigh approximately 9 g of extract ,while filtering through a 0.2 micron membrane filter, onto weighing boat and record weight.
2. Transfer contents to digestion tube and rinse boat three times with distilled water.
3. Add two Kjeltabs to tube.
4. Add 15 ml concentrated sulfuric acid to tube.
5. Place in digester and secure the water aspirator.
6. Digest until a clear, green solution is obtained (app. 1 h and 45 min).
7. Add 75 ml distilled water carefully to each digestion tube when it is cool enough to not boil over.
8. Proceed with distillation.

Protein in Cheese Digestion Method

1. Weigh out approximately 2 g of grated cheese on to filter paper and record weight. (This is what the researcher did, but this was about 10 times too much protein to titrate with 0.1 M HCl. 0.2 g would be much better) .
2. Transfer contents to digestion tube and rinse boat three times with distilled water.
3. Add two Kjeltabs to tube.
4. Add 15 ml concentrated sulfuric acid to tube.
5. Place in digester and secure the water aspirator.
6. Digest until a clear, green solution is obtained (app. 2 h). It may be necessary to rinse burnt filter paper from the neck back into the flask with distilled water and to continue digesting until the green solution is reached again.
7. Proceed with distillation

Distillation Procedure

1. Turn on the distillation unit and prepare steam generator and NaOH dispenser as per the units instructions.
2. Secure the digestion flask in position.
3. Measure 25 ml Kjeldahl boric acid with color indicator solution into an 250 ml Erlenmeyer flask and position in distillation unit with the outlet tube from the still under the fluid level in the flask.
4. Close the shield, dispense the 50 ml NaOH, and turn on the steam generator. Let distillation proceed for 5 min or until 150 ml of distillate has been collected.
5. Add a stir-bar to the distillate flask and titrate with standardized 0.1 N HCl for the extract and 1 N HCl for the protein until the green solution turns to a neutral gray.

6. Calculate the % nitrogen as (1.401 * (ml titrant of sample - ml titrant of blank) * N HCl) / g of sample. For the percent protein calculation, multiply the % nitrogen by the conversion factor for dairy protein, 6.38.

APPENDIX D: SALT DETERMINATION METHOD

Materials

Orion EA 940 microprocessor ionanalyzer

Orion 94-17 chloride ion-selective electrode

Orion 90-02 Double Junction Reference Electrode

Magnetic stirrer/hot plate for extraction

Magnetic stirrer for measurement

250 ml glass beakers

100 ml and 1000 ml volumetric flask

1 and 10 ml volumetric pipettes

watch glasses

chopper for shredding cheese

Reagents/Solutions

Reagent grade, concentrated nitric acid

Orion reference electrode filling solutions (Cat. Nos. 900002 and 900003)

Extracting Solution -Add approximately 800 ml of distilled water to a 1L volumetric flask. Carefully add 6.3 ml nitric acid to the volumetric flask. Swirl to mix. Add water to the 1L mark.(0.1M solution)

Cl- as %NaCl Standard- Weigh out 5.0 g reagent grade NaCl and place in a 100 ml volumetric flask. Dissolve and dilute to the mark using extracting solution. (5% w/v). Transfer 10 ml of this solution to another 100 ml volumetric flask and bring to 100 ml with extracting solution (0.5% w/v)

Reagent grade acetone

Distilled water

Method

Setup

1. Turn on ionanalyzer and let warm up for 30 min.
2. Prepare sufficient extracting solution (100 ml/analysis) for the day.
3. Prepare electrodes.

   Reference Electrode

   Inner Chamber - Use Orion No. 900002 colored reference electrode filling solution. Unscrew the cap and slide cap and spring up the cable. Push down on the top of inner chamber until cone at bottom end can be grasped using a tissue. Grasp cone and pull inner chamber free of outer sleeve. Slide rubber sleeve at top of inner chamber down to uncover filling hole. Using the flip-spout bottle, fill inner chamber up to fill hole and slide rubber sleeve back up. If having trouble filling inner chamber, add some solution and shake electrode down like a clinical thermometer, repeat until filled. Wipe excess filling solution off inner chamber surfaces and slide inner chamber completely up into outer sleeve. Place the spring back on inner chamber and screw cap on finger-tight.

   Outer Chamber: Fill with Orion No. 900003 reference electrode filling solution. Use a flip top bottle and fill through outer filling hole. Tip the electrode to moisten the green O-ring on the electrode body. Holding the electrode by the cap in one hand, push the outer sleeve up into the cap with the other hand, allowing the filling solution to wet the inner cone. Release the sleeve, check to see that the end of the sleeve is flush with the bottom surface of the cone, and fill the outer chamber up to the filling hole. If the

sleeve does not return to the correct position, gently push it down into place.

Calibration

1. Add 1 ml of each standard to 250 ml beakers. Add 100 ml of extracting solution to each of these beakers.
2. Rinse the electrodes and immerse them in the solution.
3. Follow the instructions for a 2 point calibration and calibrate for 5 and 0.5%.

Sample Extraction and Analysis

1. Weigh out 1.00 g(±0.01g) finely grated cheese into a 250 ml beaker. Add 100 ml extracting solution and a stirring bar. Cover with a watch glass.
2. Place the covered beaker on a stirrer/hot plate and heat to boiling while stirring gently. Maintain gentle boil for 20 min.
3. Remove beaker and cool to room temperature. Don't let the fat layer solidify though.
4. Return beaker to the magnetic stirrer and stir gently.
5. Immerse the electrodes in the sample, being careful to avoid the fat layer as much as possible. Wait for the reading to stabilize (about 2 min) and record the sample concentration as %NaCl.
6. After each sample, wash the electrodes with acetone. Wipe the chloride electrode membrane gently with an acetone-wet tissue. Rinse with distilled water and blot dry.

APPENDIX E: CHEESE ASHING PROCEDURE

Materials

Crucibles - soaked in aqua regia, numbered, dried, and cooled in a dessicator

Muffle furnace at approximately 450°C

Method

1. Weigh crucible and record weight.
2. Weigh approximately 3 - 5 g grated cheese into crucible and record weight
3. Dry samples in atmospheric oven.
4. Ignite cheese over a Bunsen burner. Remove from flame if cheese ignites.
5. Complete ignition in furnace until samples are white (overnight).
6. Cool and weigh.

APPENDIX F: DEMOGRAPHICS RESULTS

Demographics results for all panels combined showing the percent of respondents answering the following questions. 162 panelists participated.

1. What is your age group? (please check one)

   18-24 years old 48% 25-34 years old 30% 35-44 years old 15%

   45-54 years old 1% 55-64 years old 4% Over 64 years old

2. What is your sex? Female 40% Male 60%

3. What do you consider yourself to be? (please check one)

   White 75% Black 5% Spanish/Hispanic 5%

   Asian 10% Other (please specify) 5%

4. What is your marital status? (please check one)

   Never married 66% Married 31%

   Separated, divorced or widowed 3%

5. Level of education achieved? (Please check one)

   0 Less than 7 years of school

   0 Junior high school

   0 Some high school

   1% Completed high school or equivalent

   43% Currently attending college (not yet completed first degree)

   19% Completed undergraduate degree

   38% Graduate or professional school completed (master's, Ph.D., law, medicine, etc.)

6. Please check one which best applies to you:

24%	Employed full-time	0	Homemaker
17%	Employed part-time	57%	Student
2%	Unemployed	0	Disabled
0	Retired		

7. What was the approximate level of you household income before taxes last year? (please check one)

15%	under $9,999	6%	$40,000 to $49,999
31%	$10,000 to $19,000	5%	$50,000 to $59,999
12%	$20,000 to $29,999	4%	$60,000 to $69,999
7%	$30,000 to $39,999	9%	$70,000 and over

PLEASE PROVIDE GENERAL INFORMATION ABOUT YOUR CONSUMPTION OF CHEESE PRODUCTS.

1. What type of cheese do you eat on a weekly basis? (you may check more than one)

69%	Cheddar	2%	Latin American
44%	Mozzarella	39%	American/Process cheese
24%	Swiss	25%	Cream Cheese
17%	Monterey Jack	15%	Other

2. What type of Cheddar cheese do you buy?

22%	Mild
38%	Medium
18%	Sharp
9%	X-tra Sharp
13%	I don't buy Cheddar Cheese

3. Do you purchase reduced fat cheese products?

23% Never

25% Rarely

31% Sometimes

16% Often

5% Only purchase reduced fat cheese products

4. What is **the most** important factor in your cheese purchasing decision?

23% Price

2% Brand name

63% Taste

11% Nutritional attributes of the product

Thank you!

APPENDIX G: CONSUMER FLAVOR DIFFERENCE TEST FORM

Flavor Analysis

Sample #'s _____ _____

Instructions:

1. Please examine the two samples of cheese for flavor. Begin with the left sample.

2. Please check the statement you most agree with.

_____ The samples taste different.

_____ The samples taste the same.

If you answered that they are different, which sample has the more intense flavor?

_____

Comments:

APPENDIX H: CONSUMER TEXTURE DIFFERENCE TEST FORM

Texture Analysis

Sample #'s _____ _____

Instructions:

1. Please examine the two samples of cheese for texture. Begin with the left sample.

2. Please check the statement you most agree with.

_____ The samples have a different texture.

_____ The samples have the same texture.

If you answered that they are different, which sample has the softer texture? _____

Comments:

APPENDIX I: CONSUMER PREFERENCE TEST FORM

Sample No. _____

Instructions: Please check the statement you most agree with.

1. How would you rate the "**flavor**" of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9

2. How would you rate the "**texture**" of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9

3. Overall, how well do you "**like**" this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9

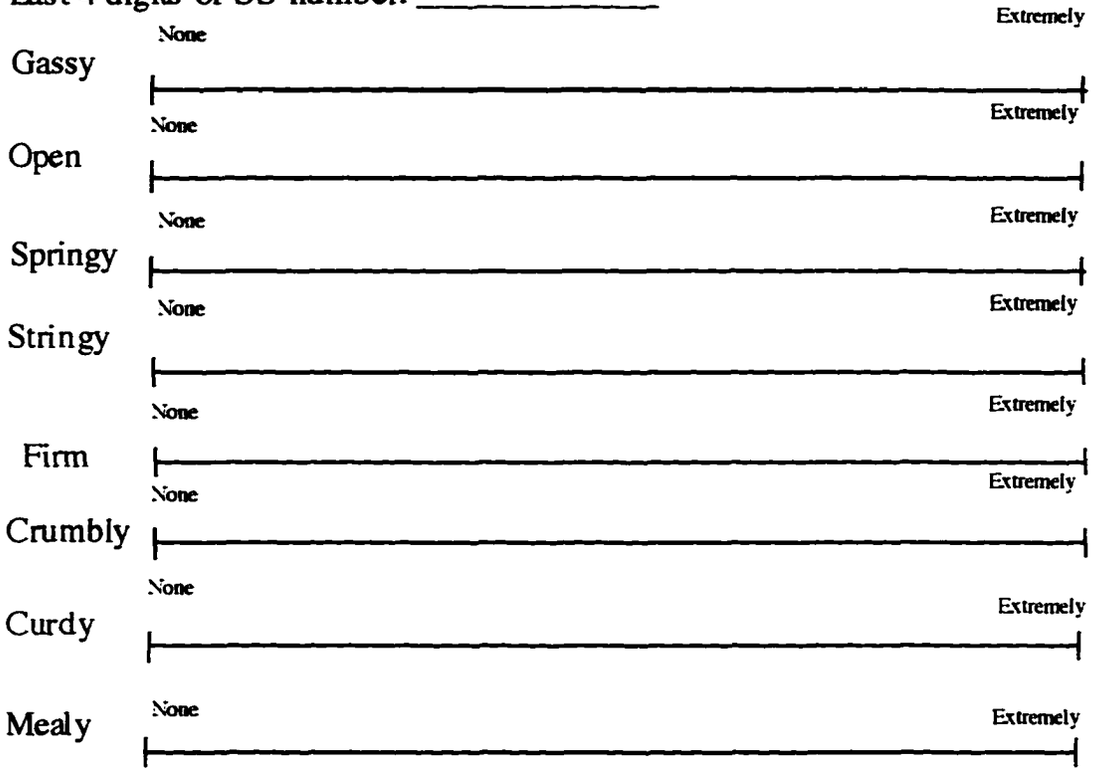
4. Is this product **acceptable**? Yes [ ] No [ ]

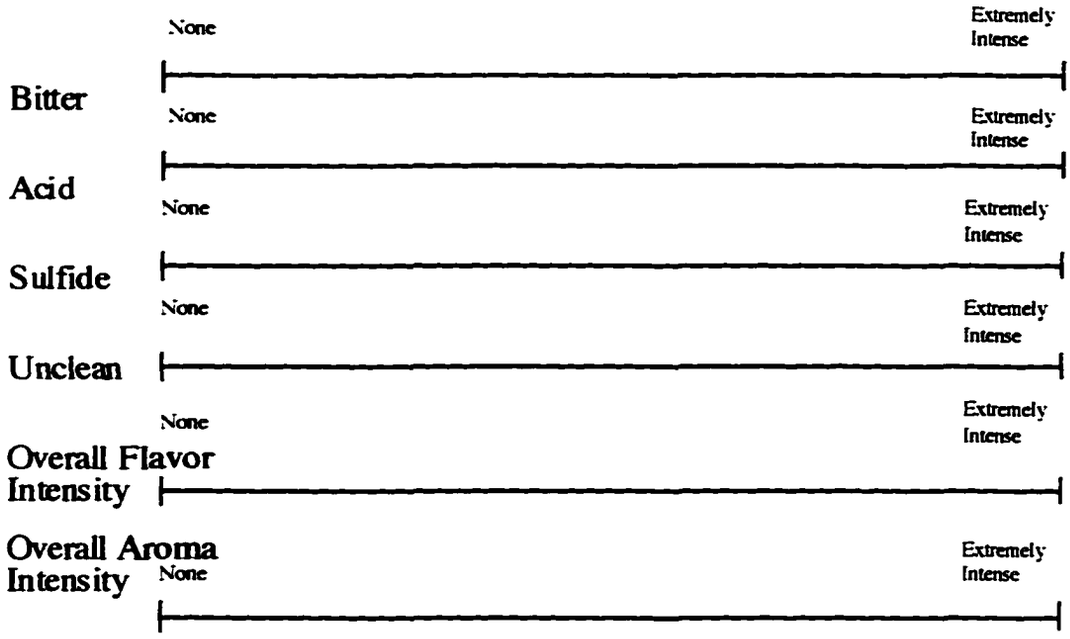
5. Given the fact that this cheese has much less fat than normal cheddar cheese, would you buy this product if it were commercially available? Yes [ ] No [ ]

APPENDIX J: EXPERIENCED PANEL SENSORY EVALUATION FORMS

Sample No. _____ Date: _____

Last 4 digits of SS number: _____





APPENDIX K: STATISTICAL RESULTS

Moisture

Class	Levels	Values
REP	3	1 2 3
WP	2	b c
SP	2	n w

Number of observations in data set = 96

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	55.4310857	5.0391896	19	0.0001
Error	84	22.2736675	0.2651627		
Corrected Total	95				

R-Square	C.V.	Root MSE	Y Mean
0.71335	1.026629	0.51494	50.1583

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	10.418471	5.2092355	19.65	0.0001
WP	1	33.6212537	33.6212537	126.79	0.0001
Rep * WP	2	1.0526738	0.5263369	1.98	0.1438
SP	1	8.2144773	8.2144773	30.98	0.0001
WP * SP	1	0.0078935	0.0078935	0.03	0.8634
Rep * WP * SP	4	2.1163164	0.5290791	2	0.1026

Test of Hypothesis using Type III MS for Rep * WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F

WP	1	33.6212537	33.6212537	63.88	0.0153

Fatty Acids

Class Level Information

Class	Levels	Values
REP	3	1 2 3
TIME	4	0 1 2 4
WP	2	b c
SP	2	n w

Number of observations in data set = 91

General Linear Models Procedure

Dependent Variable: C2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	433790525	22831080	5.47	0.0001
Error	71	296223513	4172162		
Corrected Total	90	730014038			

R-Square	C.V.	Root MSE	C2 Mean
0.594222	42.25833	2042.59	4833.57

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	30045446	15022723	3.60	0.0324
WP	1	50809594	50809594	12.18	0.0008
REP*WP	2	16887960	8443980	2.02	0.1397
SP	1	630421	630421	0.15	0.6986
WP*SP	1	5882584	5882584	1.41	0.2390
TIME	3	255496514	85165505	20.41	0.0001
TIME*SP	3	26690738	8896913	2.13	0.1037

TIME*WP*SP	6	22414336	3735723	0.90	0.5032

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	50809594.0	50809594.0	6.02	0.1337

Dependent Variable: C4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	15354.7335	808.1439	3.50	0.0001
Error	71	16411.2188	231.1439		
Corrected Total	90	31765.9523			

R-Square	C.V.	Root MSE	C4 Mean
0.483371	18.58312	15.2034	81.8131

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	2290.28831	1145.14415	4.95	0.0097
WP	1	1111.88715	1111.88715	4.81	0.0316
REP*WP	2	1540.13354	770.06677	3.33	0.0414
SP	1	173.72878	173.72878	0.75	0.3889
WP*SP	1	169.73113	169.73113	0.73	0.3944
TIME	3	7767.32075	2589.10692	11.20	0.0001
TIME*SP	3	135.50182	45.16727	0.20	0.8992
TIME*WP*SP	6	1479.69292	246.61549	1.07	0.3905

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	1111.88715	1111.88715	1.44	0.3525

Dependent Variable: C6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	419.595820	22.083991	1.34	0.1897
Error	71	1174.039837	16.535772		
Corrected Total	90	1593.635657			

R-Square	C.V.	Root MSE	C6 Mean
0.263295	77.64577	4.06642	5.23714

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	34.691560	17.345780	1.05	0.3557
WP	1	10.789224	10.789224	0.65	0.4219
REP*WP	2	47.126597	23.563298	1.42	0.2473
SP	1	12.961849	12.961849	0.78	0.3789
WP*SP	1	19.798155	19.798155	1.20	0.2776
TIME	3	117.242009	39.080670	2.36	0.0784
TIME*SP	3	49.405848	16.468616	1.00	0.3999
TIME*WP*SP	6	142.924554	23.820759	1.44	0.2114

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	10.7892235	10.7892235	0.46	0.5684

Dependent Variable: C8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	19.4671037	1.0245844	0.74	0.7651
Error	71	98.2484788	1.3837814		
Corrected Total	90	117.7155824			

R-Square	C.V.	Root MSE	C8 Mean		
0.165374	334.3134	1.17634	0.35187		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	4.65323564	2.32661782	1.68	0.1934
WP	1	1.02319124	1.02319124	0.74	0.3927
REP*WP	2	0.56983164	0.28491582	0.21	0.8144
SP	1	0.01160577	0.01160577	0.01	0.9273
WP*SP	1	0.01097840	0.01097840	0.01	0.9293
TIME	3	3.37354336	1.12451445	0.81	0.4911
TIME*SP	3	3.39973917	1.13324639	0.82	0.4877
TIME*WP*SP	6	5.33075051	0.88845842	0.64	0.6962

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	1.02319124	1.02319124	3.59	0.1986

Dependent Variable: C10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	2766.73902	145.61784	2.00	0.0190
Error	71	5168.28525	72.79275		
Corrected Total	90	7935.02427			

R-Square	C.V.	Root MSE	C10 Mean		
0.348674	125.6982	8.53187	6.78758		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	415.276880	207.638440	2.85	0.0643
WP	1	0.006112	0.006112	0.00	0.9927
REP*WP	2	522.650318	261.325159	3.59	0.0327

SP	1	191.387991	191.387991	2.63	0.1093
WP*SP	1	5.940634	5.940634	0.08	0.7760
TIME	3	720.122166	240.040722	3.30	0.0253
TIME*SP	3	403.470888	134.490296	1.85	0.1464
TIME*WP*SP	6	600.449983	100.074997	1.37	0.2367

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	0.00611204	0.00611204	0.00	0.9966

Dependent Variable: C12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	614.439134	32.338902	0.92	0.5657
Error	71	2506.141334	35.297765		
Corrected Total	90	3120.580468			

R-Square	C.V.	Root MSE	C12 Mean
0.196899	62.35782	5.94119	9.52758

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	31.111634	15.555817	0.44	0.6453
WP	1	31.365211	31.365211	0.89	0.3491
REP*WP	2	185.795475	92.897737	2.63	0.0790
SP	1	8.889081	8.889081	0.25	0.6173
WP*SP	1	20.842871	20.842871	0.59	0.4448
TIME	3	88.098963	29.366321	0.83	0.4807
TIME*SP	3	36.405010	12.135003	0.34	0.7937
TIME*WP*SP	6	154.207065	25.701178	0.73	0.6284

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	31.3652114	31.3652114	0.34	0.6200

Dependent Variable: C14

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	23450.9310	1234.2595	2.38	0.0045
Error	71	36748.9636	517.5910		
Corrected Total	90	60199.8947			

R-Square	C.V.	Root MSE	C14 Mean
0.389551	80.97322	22.7506	28.0965

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	4937.75124	2468.87562	4.77	0.0114
WP	1	475.99949	475.99949	0.92	0.3408
REP*WP	2	2632.06495	1316.03248	2.54	0.0858
SP	1	1179.31583	1179.31583	2.28	0.1356
WP*SP	1	607.28382	607.28382	1.17	0.2824
TIME	3	5922.23317	1974.07772	3.81	0.0136
TIME*SP	3	3645.30862	1215.10287	2.35	0.0799
TIME*WP*SP	6	3152.19217	525.36536	1.02	0.4226

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	475.999491	475.999491	0.36	0.6087

Dependent Variable: C16

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	65062.8092	3424.3584	2.10	0.0129

Error	71	115522.4059	1627.0761	
Corrected Total	90	180585.2151		

R-Square	C.V.	Root MSE	C16 Mean
0.360289	34.06487	40.3370	118.412

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	9377.7746	4688.8873	2.88	0.0626
WP	1	1498.9587	1498.9587	0.92	0.3404
REP*WP	2	18606.3790	9303.1895	5.72	0.0050
SP	1	143.1304	143.1304	0.09	0.7676
WP*SP	1	812.7207	812.7207	0.50	0.4820
TIME	3	19272.8505	6424.2835	3.95	0.0116
TIME*SP	3	2031.6245	677.2082	0.42	0.7419
TIME*WP*SP	6	6822.7980	1137.1330	0.70	0.6513

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	1498.95866	1498.95866	0.16	0.7270

Dependent Variable: C18

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	687188.896	36167.837	4.57	0.0001
Error	71	562498.950	7922.520		
Corrected Total	90	1249687.846			

R-Square	C.V.	Root MSE	C18 Mean
0.549888	45.46164	89.0085	195.788

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	279362.330	139681.165	17.63	0.0001

WP	1	56208.333	56208.333	7.09	0.0096
REP*WP	2	83915.797	41957.899	5.30	0.0072
SP	1	31310.585	31310.585	3.95	0.0507
WP*SP	1	13932.138	13932.138	1.76	0.1891
TIME	3	70651.389	23550.463	2.97	0.0374
TIME*SP	3	14112.444	4704.148	0.59	0.6211
TIME*WP*SP	6	104774.977	17462.496	2.20	0.0524

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	56208.3330	56208.3330	1.34	0.3666

Dependent Variable: C18:1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	176619.225	9295.749	2.20	0.0091
Error	71	300394.311	4230.906		
Corrected Total	90	477013.536			

R-Square	C.V.	Root MSE	C181 Mean
0.370260	29.22392	65.0454	222.576

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	589.014	294.507	0.07	0.9328
WP	1	772.765	772.765	0.18	0.6704
REP*WP	2	5646.009	2823.004	0.67	0.5163
SP	1	624.862	624.862	0.15	0.7019
WP*SP	1	512.336	512.336	0.12	0.7289
TIME	3	153366.506	51122.169	12.08	0.0001
TIME*SP	3	1361.655	453.885	0.11	0.9556

TIME*WP*SP	6	15509.321	2584.887	0.61	0.7208

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	772.765087	772.765087	0.27	0.6530

Moisture in Non-fat Substance

General Linear Models Procedure

Class Level Information

Class	Levels	Values
REP	3	1 2 3
WP	2	b c

Number of observations in data set = 12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	9.44665374	1.88933075	14.66	0.0026
Error	6	0.77335853	0.12889309		
Corrected Total	11	10.22001227			

R-Square	C.V.	Root MSE	MNFS Mean
0.924329	0.644159	0.35902	55.7342

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	4.56438710	2.28219355	17.71	0.0030
WP	1	4.75120322	4.75120322	36.86	0.0009
REP*WP	2	0.13106341	0.06553171	0.51	0.6252

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	4.75120322	4.75120322	72.50	0.0135

Fat in Dry Matter

General Linear Models Procedure

Class Level Information

Class	Levels	Values
REP	3	1 2 3
WP	2	b c

Number of observations in data set = 12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	18.6236900	3.7247380	8.53	0.0106
Error	6	2.6186090	0.4364348		
Corrected Total	11	21.2422989			

R-Square	C.V.	Root MSE	Y Mean
0.876727	3.292042	0.66063	20.0675

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	15.1343596	7.5671798	17.34	0.0032
WP	1	0.2753765	0.2753765	0.63	0.4573
REP*WP	2	3.2139538	1.6069769	3.68	0.0905

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	0.27537649	0.27537649	0.17	0.7191

Microbiology

General Linear Models Procedure

Class Level Information

Class	Levels	Values

REP	3	1 2 3
TIME	3	1 2 4
WP	2	b c
SP	2	n w

Number of observations in data set = 72

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	27.9906190	1.8660413	10.96	0.0001
Error	56	9.5325168	0.1702235		
Corrected Total	71	37.5231358			

R-Square	C.V.	Root MSE	Y Mean
0.745956	5.819769	0.41258	7.08931

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	11.4927462	5.7463731	33.76	0.0001
WP	1	2.7003846	2.7003846	15.86	0.0002
REP*WP	2	4.3184329	2.1592164	12.68	0.0001
SP	1	0.0649026	0.0649026	0.38	0.5394
WP*SP	1	0.0104096	0.0104096	0.06	0.8056
TIME	2	7.9725912	3.9862956	23.42	0.0001
TIME*SP	2	0.3486947	0.1743474	1.02	0.3657
TIME*WP*SP	4	1.0824572	0.2706143	1.59	0.1897

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	2.70038457	2.70038457	1.25	0.3797

Instron Texture Analysis

General Linear Models Procedure

Class Level Information

Class	Levels	Values
REP	3	1 2 3
TIME	4	0 1 2 4
WP	2	b c
SP	2	n w

Number of observations in data set = 191

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	6.96011101	0.36632163	17.87	0.0001
Error	171	3.50587442	0.02050219		
Corrected Total	190	10.46598544			

R-Square	C.V.	Root MSE	Y Mean
0.665022	25.06675	0.14319	0.57122

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	3.23344799	1.61672399	78.86	0.0001
WP	1	1.81970219	1.81970219	88.76	0.0001
REP*WP	2	0.37357772	0.18678886	9.11	0.0002
SP	1	0.11661171	0.11661171	5.69	0.0182
WP*SP	1	0.13998970	0.13998970	6.83	0.0098
TIME	3	0.39006522	0.13002174	6.34	0.0004
TIME*SP	3	0.25747507	0.08582502	4.19	0.0069
TIME*WP*SP	6	0.61006132	0.10167689	4.96	0.0001

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	1.81970219	1.81970219	9.74	0.0891

Peptide Extracts

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TIME	4	0 1 2 4
REP	3	1 2 3
WP	2	b c
SP	2	n w

Number of observations in data set = 96

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	0.02867467	0.00136546	142.69	0.0001
Error	74	0.00070814	0.00000957		
Corrected Total	95	0.02938282			

R-Square	C.V.	Root MSE	Y Mean
0.975899	8.412258	0.00309	0.03677

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	0.00017127	0.00008563	8.95	0.0003
WP	1	0.00013735	0.00013735	14.35	0.0003
REP*WP	2	0.00004623	0.00002311	2.42	0.0964
SP	1	0.00001527	0.00001527	1.60	0.2105
REP*SP	2	0.00002867	0.00001433	1.50	0.2303
WP*SP	1	0.00009870	0.00009870	10.31	0.0020
TIME	3	0.02800651	0.00933550	975.55	0.0001
TIME*SP	3	0.00007928	0.00002643	2.76	0.0480
TIME*WP*SP	6	0.00009141	0.00001523	1.59	0.1615

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	0.00013735	0.00013735	5.94	0.1350

Total Protein

General Linear Models Procedure

Class Level Information

Class	Levels	Values
REP	3	1 2 3
WP	2	B C
SP	2	N W

Number of observations in data set = 20

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	28.0747862	2.5522533	22.90	0.0001
Error	8	0.8916320	0.1114540		
Corrected Total	19	28.9664182			

R-Square	C.V.	Root MSE	Y Mean
0.969218	1.012036	0.33385	32.9877

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	10.2170701	5.1085350	45.84	0.0001
WP	1	8.7633911	8.7633911	78.63	0.0001
REP*WP	2	1.3556047	0.6778023	6.08	0.0248
SP	1	1.7409780	1.7409780	15.62	0.0042
WP*SP	1	0.0330245	0.0330245	0.30	0.6010
REP*WP*SP	4	1.4707683	0.3676921	3.30	0.0708

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WP	1	8.76339112	8.76339112	12.93	0.0694

Salt in Moisture

General Linear Models Procedure

Class Level Information

Class	Levels	Values
REP	3	1 2 3
WP	2	b c
SP	2	n w

Number of observations in data set = 24

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	17.9157365	1.6287033	3.58	0.0190
Error	12	5.4613377	0.4551115		
Corrected Total	23	23.3770743			

R-Square	C.V.	Root MSE	Y Mean
0.766381	17.40825	0.67462	3.87529

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	2	6.61743709	3.30871854	7.27	0.0085
WP	1	0.50569327	0.50569327	1.11	0.3126
REP*WP	2	3.02656843	1.51328422	3.33	0.0710
SP	1	1.56931442	1.56931442	3.45	0.0880
WP*SP	1	0.31843177	0.31843177	0.70	0.4192
REP*WP*SP	4	5.87829154	1.46957289	3.23	0.0513

Tests of Hypotheses using the Type III MS for REP*WP as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F

WP	1	0.50569327	0.50569327	0.33	0.6216

Experienced Sensory Panel

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TIME	6	11 13 15 17 6 8
SAMPLE	2	b n
PANEL	8	1068 3207 3772 4905 618 679 7299 9882

Number of observations in data set = 88

Dependent Variable: GASSY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	701.491610	25.981171	3.81	0.0001
Error	60	409.643598	6.827393		
Corrected Total	87	1111.135208			

R-Square	C.V.	Root MSE	GASSY Mean
0.631329	76.70470	2.61293	3.40648

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	3.212078	3.212078	0.47	0.4954
PANEL(SAMPLE)	16	656.997627	41.062352	6.01	0.0001
TIME	5	8.357892	1.671578	0.24	0.9408
TIME*SAMPLE	5	30.273710	6.054742	0.89	0.4957

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	3.21207761	3.21207761	0.08	0.7833

Dependent Variable: OPEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	642.050402	23.779645	4.26	0.0001
Error	60	334.814684	5.580245		
Corrected Total	87	976.865086			

R-Square	C.V.	Root MSE	OPEN Mean
0.657256	59.14709	2.36225	3.99386

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	24.141563	24.141563	4.33	0.0418
PANEL(SAMPLE)	16	501.141601	31.321350	5.61	0.0001
TIME	5	77.864799	15.572960	2.79	0.0248
TIME*SAMPLE	5	30.775724	6.155145	1.10	0.3684

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	24.1415633	24.1415633	0.77	0.3930

Dependent Variable: SPRINGY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	670.848201	24.846230	4.24	0.0001
Error	60	351.855543	5.864259		
Corrected Total	87	1022.703744			

R-Square	C.V.	Root MSE	SPRING Mean
0.655956	41.43632	2.42162	5.84420

Source	DF	Type III SS	Mean Square	F Value	Pr > F

SAMPLE	1	18.200904	18.200904	3.10	0.0832
PANEL(SAMPLE)	16	319.239448	19.952466	3.40	0.0003
TIME	5	270.512306	54.102461	9.23	0.0001
TIME*SAMPLE	5	30.242151	6.048430	1.03	0.4075

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	18.2009041	18.2009041	0.91	0.3537

Dependent Variable: STRINGY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	649.801760	24.066732	4.36	0.0001
Error	60	330.892484	5.514875		
Corrected Total	87	980.694244			

R-Square	C.V.	Root MSE	STRING Mean
0.662594	52.75231	2.34838	4.45170

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.624953	0.624953	0.11	0.7376
PANEL(SAMPLE)	16	496.071550	31.004472	5.62	0.0001
TIME	5	87.041929	17.408386	3.16	0.0135
TIME*SAMPLE	5	25.480810	5.096162	0.92	0.4718

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.62495277	0.62495277	0.02	0.8889

Dependent Variable: FIRM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	520.981022	19.295593	1.95	0.0161
Error	60	593.062449	9.884374		
Corrected Total	87	1114.043472			

R-Square	C.V.	Root MSE	FIRM Mean
0.467649	54.10732	3.14394	5.81057

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	2.882419	2.882419	0.29	0.5912
PANEL(SAMPLE)	16	393.468822	24.591801	2.49	0.0057
TIME	5	82.203915	16.440783	1.66	0.1574
TIME*SAMPLE	5	4.289423	0.857885	0.09	0.9941

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	2.88241914	2.88241914	0.12	0.7365

Dependent Variable: CRUMBLY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	383.490030	14.203334	2.50	0.0016
Error	60	340.902169	5.681703		
Corrected Total	87	724.392199			

R-Square	C.V.	Root MSE	CRUMBLY Mean
0.529396	91.06127	2.38363	2.61761

Source	DF	Type III SS	Mean Square	F Value	Pr > F

SAMPLE	1	36.880959	36.880959	6.49	0.0134
PANEL(SAMPLE)	16	299.291787	18.705737	3.29	0.0004
TIME	5	36.092153	7.218431	1.27	0.2885
TIME*SAMPLE	5	8.445519	1.689104	0.30	0.9125

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	36.8809590	36.8809590	1.97	0.1794

Dependent Variable: CURDY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	543.918885	20.145144	5.52	0.0001
Error	60	218.886778	3.648113		
Corrected Total	87	762.805662			

R-Square	C.V.	Root MSE	CURDY Mean
0.713050	72.52030	1.91000	2.63375

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	6.169426	6.169426	1.69	0.1984
PANEL(SAMPLE)	16	449.518471	28.094904	7.70	0.0001
TIME	5	50.766683	10.153337	2.78	0.0251
TIME*SAMPLE	5	12.402070	2.480414	0.68	0.6404

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	6.16942560	6.16942560	0.22	0.6457

Dependent Variable: MEALY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	315.018110	11.667337	3.89	0.0001
Error	60	179.762521	2.996042		
Corrected Total	87	494.780632			

R-Square	C.V.	Root MSE	MEALY Mean
0.636682	85.77536	1.73091	2.01795

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	1.442890	1.442890	0.48	0.4904
PANEL(SAMPLE)	16	268.364877	16.772805	5.60	0.0001
TIME	5	27.086269	5.417254	1.81	0.1249
TIME*SAMPLE	5	11.941779	2.388356	0.80	0.5560

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	1.44289043	1.44289043	0.09	0.7731

Dependent Variable: BITTER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	198.277092	7.343596	1.66	0.0526
Error	60	265.545467	4.425758		
Corrected Total	87	463.822559			

R-Square	C.V.	Root MSE	BITTER Mean
0.427485	92.70399	2.10375	2.26932

Source	DF	Type III SS	Mean Square	F Value	Pr > F

SAMPLE	1	9.534982	9.534982	2.15	0.1474
PANEL(SAMPLE)	16	106.852372	6.678273	1.51	0.1267
TIME	5	66.426410	13.285282	3.00	0.0175
TIME*SAMPLE	5	5.808236	1.161647	0.26	0.9318

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	9.53498241	9.53498241	1.43	0.2495

Dependent Variable: ACID

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	350.987393	12.999533	3.18	0.0001
Error	60	245.570897	4.092848		
Corrected Total	87	596.558290			

R-Square	C.V.	Root MSE	ACID Mean
0.588354	44.34477	2.02308	4.56216

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	28.811347	28.811347	7.04	0.0102
PANEL(SAMPLE)	16	232.769517	14.548095	3.55	0.0002
TIME	5	64.274477	12.854895	3.14	0.0139
TIME*SAMPLE	5	17.566562	3.513312	0.86	0.5144

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	28.8113465	28.8113465	1.98	0.1785

Dependent Variable: SULFIDE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	216.453753	8.016806	1.56	0.0770
Error	60	308.182229	5.136370		
Corrected Total	87	524.635982			

R-Square	C.V.	Root MSE	SULFIDE Mean
0.412579	77.73595	2.26636	2.91545

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.906376	0.906376	0.18	0.6759
PANEL(SAMPLE)	16	168.687973	10.542998	2.05	0.0235
TIME	5	34.426906	6.885381	1.34	0.2597
TIME*SAMPLE	5	7.592958	1.518592	0.30	0.9135

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.90637605	0.90637605	0.09	0.7731

Dependent Variable: UNCLEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	402.047498	14.890648	2.85	0.0004
Error	60	313.542647	5.225711		
Corrected Total	87	715.590144			

R-Square	C.V.	Root MSE	UNCLEAN Mean
0.561840	107.5353	2.28598	2.12580

Source	DF	Type III SS	Mean Square	F Value	Pr > F

SAMPLE	1	22.143063	22.143063	4.24	0.0439
PANEL(SAMPLE)	16	266.610328	16.663146	3.19	0.0006
TIME	5	58.774309	11.754862	2.25	0.0608
TIME*SAMPLE	5	33.328414	6.665683	1.28	0.2863

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	22.1430634	22.1430634	1.33	0.2659

Dependent Variable: FLAVOR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	271.507224	10.055823	1.89	0.0209
Error	60	319.336920	5.322282		
Corrected Total	87	590.844144			

R-Square	C.V.	Root MSE	FLAVOR Mean
0.459524	44.93407	2.30701	5.13420

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	13.833539	13.833539	2.60	0.1122
PANEL(SAMPLE)	16	102.007648	6.375478	1.20	0.2963
TIME	5	104.580480	20.916096	3.93	0.0038
TIME*SAMPLE	5	22.860167	4.572033	0.86	0.5139

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	13.8335391	13.8335391	2.17	0.1601

Dependent Variable: AROMA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	191.946143	7.109116	1.92	0.0188
Error	60	222.702144	3.711702		
Corrected Total	87	414.648286			

R-Square	C.V.	Root MSE	AROMA Mean
0.462913	53.75701	1.92658	3.58386

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.435279	0.435279	0.12	0.7332
PANEL(SAMPLE)	16	119.568351	7.473022	2.01	0.0266
TIME	5	41.946167	8.389233	2.26	0.0598
TIME*SAMPLE	5	28.922613	5.784523	1.56	0.1857

Tests of Hypotheses using the Type III MS for PANEL(SAMPLE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SAMPLE	1	0.43527929	0.43527929	0.06	0.8124

Consumer Panel Preference

Month 2 Consumer Panel

t-Test: Paired Two Sample for Means for "Flavor"

	Buttermilk	Normal
Mean	6.466666667	6.355555556
Variance	2.254545455	3.27979798
Observations	45	45
Pearson Correlation	0.405634046	
Hypothesized Mean Difference	0	
df	44	
t Stat	0.408557921	
P(T<=t) one-tail	0.342422885	
t Critical one-tail	1.680230071	
P(T<=t) two-tail	0.68484577	
t Critical two-tail	2.0153675	

t-Test: Paired Two Sample for Means for "Texture"

	Buttermilk	Normal
Mean	6.688888889	6.2
Variance	2.037373737	2.936363636
Observations	45	45
Pearson Correlation	0.472031265	
Hypothesized Mean Difference	0	
df	44	

t Stat	2.009077202
P(T<=t) one-tail	0.025344269
t Critical one-tail	1.680230071
P(T<=t) two-tail	0.050688539
t Critical two-tail	2.0153675

t-Test: Paired Two Sample for Means for "Overall"

	Buttermilk	Normal
Mean	6.644444444	6.4
Variance	2.007070707	2.927272727
Observations	45	45
Pearson Correlation	0.388181412	
Hypothesized Mean Difference	0	
df	44	
t Stat	0.938548663	
P(T<=t) one-tail	0.176543136	
t Critical one-tail	1.680230071	
P(T<=t) two-tail	0.353086272	
t Critical two-tail	2.0153675	

Month 3 Consumer Panel

t-Test: Paired Two Sample for Means for "Flavor"

	Buttermilk	Normal
Mean	6.280701754	6.894736842

Variance	2.812656642	6.894736842
Observations	57	57
Pearson Correlation	0.447705468	
Hypothesized Mean Difference	0	
df	56	
t Stat	-2.95841726	
P(T<=t) one-tail	0.00226176	
t Critical one-tail	1.672522103	
P(T<=t) two-tail	0.004523519	
t Critical two-tail	2.003239388	

t-Test: Paired Two Sample for Means for "Texture"

	Buttermilk	Normal
Mean	6.649122807	6.824561404
Variance	1.588972431	1.790100251
Observations	57	57
Pearson Correlation	0.259313829	
Hypothesized Mean Difference	0	
df	56	
t Stat	-0.836973951	
P(T<=t) one-tail	0.203082434	
t Critical one-tail	1.672522103	
P(T<=t) two-tail	0.406164869	
t Critical two-tail	2.003239388	

t-Test: Paired Two Sample for Means for "Overall"

	Buttermilk	Normal
Mean	6.33333333	6.877192982
Variance	2.869047619	1.96679198
Observations	57	57
Pearson Correlation	0.498649861	
Hypothesized Mean Difference	0	
df	56	
t Stat	-2.61431553	
P(T<=t) one-tail	0.005732082	
t Critical one-tail	1.672522103	
P(T<=t) two-tail	0.011464165	
t Critical two-tail	2.003239388	

Month 4 Consumer Panel

t-Test: Paired Two Sample for Means for "Flavor"

	Buttermilk	Normal
Mean	5.75862069	6.724137931
Variance	2.993345433	2.308529946
Observations	58	58
Pearson Correlation	0.301245535	
Hypothesized Mean Difference	0	
df	57	
t Stat	-3.813415953	

P(T<=t) one-tail	0.000169512
t Critical one-tail	1.672028702
P(T<=t) two-tail	0.000339024
t Critical two-tail	2.002466317

t-Test: Paired Two Sample for Means for "Texture"

	Buttermilk	Normal
Mean	6.25862069	6.620689655
Variance	2.826678766	2.730792498
Observations	58	58
Pearson Correlation	0.572664572	
Hypothesized Mean Difference	0	
df	57	
t Stat	-1.789117748	
P(T<=t) one-tail	0.039454734	
t Critical one-tail	1.672028702	
P(T<=t) two-tail	0.078909468	
t Critical two-tail	2.002466317	

t-Test: Paired Two Sample for Means for "Overall"

	Buttermilk	Normal
Mean	6.017241379	6.793103448
Variance	2.929522081	2.096793708
Observations	58	58

Pearson Correlation	0.305845417
Hypothesized Mean Difference	0
df	57
t Stat	-3.153750767
P(T<=t) one-tail	0.001285943
t Critical one-tail	1.672028702
P(T<=t) two-tail	0.002571887
t Critical two-tail	2.002466317

VITA

The author, Tonya Conner Schoenfuss, was born in Orange, California, on February 13, 1967. She lived there, briefly in Healdsburg, California and then in Mission Viejo, California. In 1979 her family moved to San Jacinto, California, to start a wholesale nursery and that is when her agricultural experiences began. She was active in 4-H and FFA and showed dairy replacement heifers and dairy goats. Through these experiences she decided to attend California Polytechnic State University, San Luis Obispo, in the department of Dairy Science in the Dairy Product Technology option. While at Cal Poly she was employed by the Cal Poly Creamery and the Dairy Products Technology Center. For two summers she worked at Dannon Yogurt, Glendale, California, in their quality assurance department. In her junior year, she was selected to attend Massey University, Palmerston North, New Zealand, on the California State University's "International Program". She attended Massey University for a full academic year in the Food Technology Department. The degree of Bachelor of Science was conferred upon Tonya in September of 1989.

In July 1990, Tonya began her studies at Virginia Polytechnic Institute and State University in the department of Food Science studying under Dr. J. Russell Bishop. She studied the effect of processing milk on the ability of animal drug residue detection methods to detect contaminated samples. The degree of Master of Science was conferred upon Tonya in August 1992.

In August 1992, Tonya began her studies as a doctoral candidate at Louisiana State University in the Department of Dairy Science under the guidance of Dr. J. U. McGregor. She has been involved with the L.S.U. Food Science Club and the

Graduate Student Association. Tonya is currently a student member of the Institute of Food Technologists and the American Dairy Science Association.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Tonya Conner Schoenfuss

Major Field: Dairy Science

Title of Dissertation: The Effects of Homogenized Cream and Commercial Buttermilk Powder on Lowfat Cheddar Cheese

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 7, 1997